10/529772
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NITROANILINE-BASED ALKYLATING AGENTS AND THEIR USE AS PRODRUGS

The present invention relates to the preparation of nitroaniline-based unsymmetrical mustards, and their use as prodrugs for GDEPT (gene-dependent enzyme-prodrug therapy) and cell ablation therapy in conjunction with nitroreductase enzymes, as hypoxia-selective cytotoxins, and as anticancer agents.

BACKGROUND TO THE INVENTION

The use of tumour-selective prodrugs (relatively inactive compounds that can be selectively converted to more active compounds *in vivo*) is a valuable concept in cancer therapy.

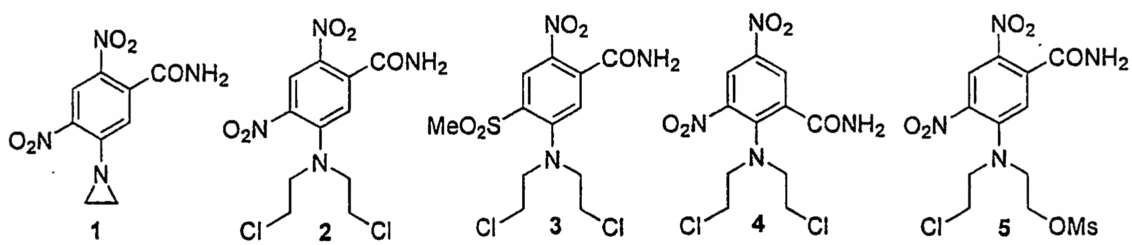
For example a prodrug may be converted into an anti-tumour agent under the influence of an enzyme that is linkable to a monoclonal antibody that will bind to a tumour associated antigen. The combination of such a prodrug with such an enzyme monoclonal/antibody conjugate represents a very powerful clinical agent. This approach to cancer therapy, often referred to as "antibody directed enzyme/prodrug therapy" (ADEPT), is disclosed in W088/07378.

A further therapeutic approach termed "virus-directed enzyme prodrug therapy" (VDEPT) has been proposed as a method for treating tumour cells in patients using prodrugs. Tumour cells are targeted with a viral vector carrying a gene encoding an enzyme capable of activating a prodrug. The gene may be transcriptionally regulated by tissue specific promoter or enhancer sequences. The viral vector enters tumour cells and expresses the enzyme, in order that a prodrug is converted to an active drug within the tumour cells (Huber et al., *Proc. Natl. Acad. Sci.* USA (1991) 88, 8039). Alternatively, non-viral methods for the delivery of genes have been used. Such methods include calcium phosphate co-precipitation, microinjection, liposomes, direct DNA uptake, and receptor-mediated DNA transfer. These are reviewed in Morgan & French, *Annu. Rev. Biochem.*, 1993, 62; 191. The term "GDEPT" (gene-directed enzyme prodrug therapy) is used to include both viral and non-viral delivery systems.

4-Nitroaromatic compounds are reduced by both mammalian and bacterial flavoprotein enzymes, which effect stepwise addition of up to six electrons. The major enzymic metabolite is usually the 4-electron species (hydroxylamine).

The present invention relates to novel nitroaniline-based unsymmetrical mustards having cytotoxic activity, to methods of preparing the novel compounds, and to the use of these compounds as prodrugs for GDEPT and for cell ablation therapy in conjunction with nitroreductase enzymes (particularly the nitro reductases encoded by the nfsB gene of *E. coli* or by *Clostridia* species), as hypoxia-selective cytotoxins, and as anticancer agents.

Both dinitrobenzamide aziridines (e.g., **1**) [Knox et al., *Cancer Met. Rev.*, 1993, 12, 195] and nitro- and dinitrobenzamide mustards (e.g., **2-4**) [Friedlos et al., *J. Med. Chem.*, 1997, 40, 1270] have been reported as substrates for the aerobic *E. coli* nitroreductase (NTR), and as specific prodrugs for GDEPT in conjunction with NTR.

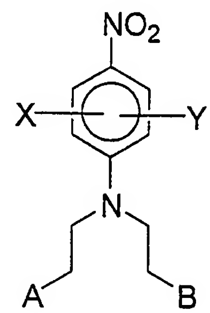


Unsymmetrical (chloro-mesylate) mustards have been reported [e.g., Marais et al., *Cancer Res.* 1996, 56, 4735], including the dinitro analogue **5** [Friedlos et al., *J. Med Chem.* 1997, 40, 1270], which was described as not sufficiently potent for a full biological evaluation to be conducted.

It is therefore an object of the invention to provide a series of unsymmetrical mustards, methods for preparing the unsymmetrical mustards that are suitable for use as prodrugs for GDEPT (gene-dependent enzyme-prodrug therapy) and cell ablation therapy in conjunction with nitroreductase enzymes, as hypoxia-selective cytotoxins, and as anticancer agents or to at least provide the public with a useful alternative.

SUMAMRY OF THE INVENTION

In a first aspect, the present invention provides a nitroaniline-based unsymmetrical mustard represented by the general formula (I);



(I)

wherein X represents one of the groups NO_2, CN, or SO_2R^1, where R^1 represents a C_{1-6}-lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and wherein when R^1 represents a tertiary amine the N-oxide derivative of the tertiary amine is further included;

Y represents one of the groups OR^2, $NHCOR^2$, $CONR^2CO_2R^3$, $CONR^2$morpholide, $CONHR^2$, $CONR^2R^3$, $CONHOR^2$, $CONHSO_2R^2$, SO_2NH_2, SO_2NHR^2 or $SO_2NR^2R^3$ wherein each R^2 and R^3 independently represent a H, C_{1-6}-lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups; and

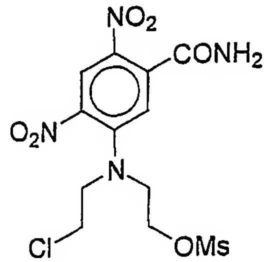
A and B each independently represent halogen, OSO_2R^4, OSO_2NH_2, OSO_2NHR^4 or $OSO_2NR^4R^5$, wherein each R^4 and R^5 independently represent a C_{1-6}-lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and wherein when each R^4 and R^5 independently represents a tertiary amine the N-oxide derivative of the tertiary amine is further included;

and pharmaceutically acceptable derivatives and salts thereof;

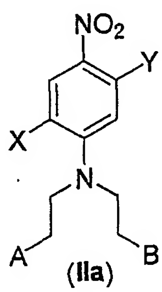
with the proviso

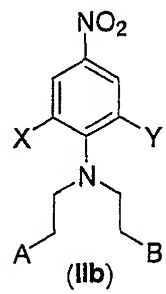
(i) that $A \neq B$ and

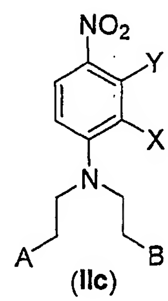
(ii) that

 is excluded.

In a preferred embodiment the nitroaniline-based unsymmetrical mustard is selected from a compound represented by one of formulae (**IIa-IIc**)





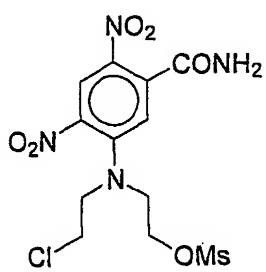


wherein X, Y, A and B are as defined above for a compound of Formula (I); and pharmaceutically acceptable derivatives and salts thereof;

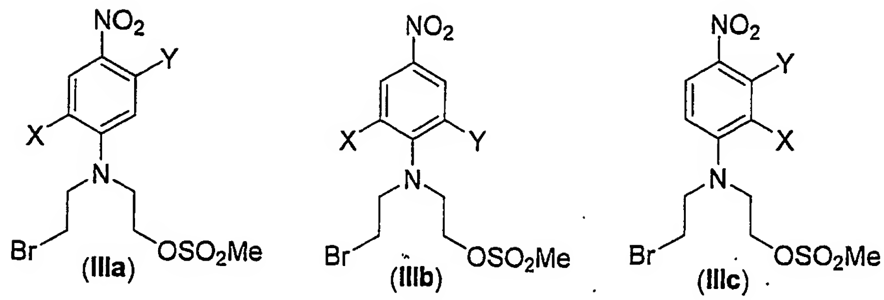
with the proviso

(i) that A ≠ B and

(iii) that

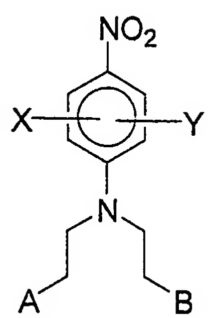
 is excluded as a compound of Formula (IIa).

In a more preferred embodiment the nitroaniline-based unsymmetrical mustard is selected from a compound represented by one of formulae (**IIIa-IIIc**)



wherein X, Y, are as defined above for a compound of Formula (I); and pharmaceutically acceptable derivatives and salts thereof.

In a second aspect of the invention there is provided a method of preparing a nitroaniline-based unsymmetrical mustard represented by the general formula (I);



(I)

wherein X represents one of the groups NO_2, CN, or SO_2R^1, where R^1 represents a C_{1-6}-lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and wherein when R^1 represents a tertiary amine the N-oxide derivative of the tertiary amine is further included;

Y represents one of the groups OR^2, $NHCOR^2$, $CONR^2CO_2R^3$, $CONR^2$morpholide, $CONHR^2$, $CONR^2R^3$, $CONHOR^2$, $CONHSO_2R^2$, SO_2NH_2, SO_2NHR^2 or $SO_2NR^2R^3$ wherein each R^2 and R^3 independently represent a H, C_{1-6}-lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups; and

A and B each independently represent halogen, OSO_2R^4, OSO_2NH_2, OSO_2NHR^4 or $OSO_2NR^4R^5$, wherein each R^4 and R^5 independently represent a C_{1-6}-lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and wherein when each R^4 and R^5 independently represents a tertiary amine the N-oxide derivative of the tertiary amine is further included;

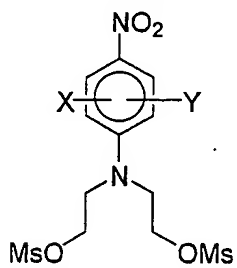
and pharmaceutically acceptable derivatives and salts thereof;

with the proviso

(i) that $A \neq B$

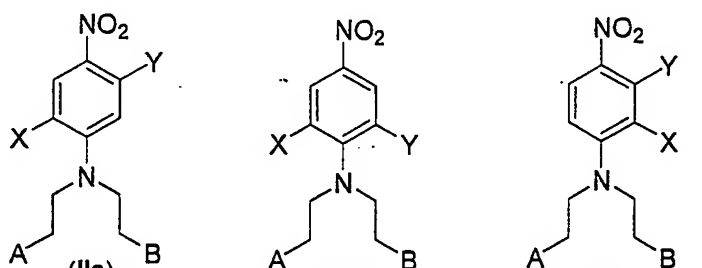
the method including the step of

(i) reacting a compound of



with an amount of an alkali metal halide in a polar solvent to give an unsymmetrical halo-mesylate compound.

In a preferred embodiment the method of preparing a nitroaniline-based unsymmetrical mustard represented by the general formula represented by one of formulae (**IIa-IIc**)



(IIa) (IIb) (IIc)

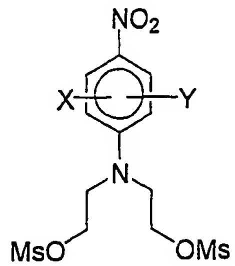
wherein X, Y, A and B are as defined above for a compound of Formula (I); and pharmaceutically acceptable derivatives and salts thereof;

with the proviso

(i) that $A \neq B$ and

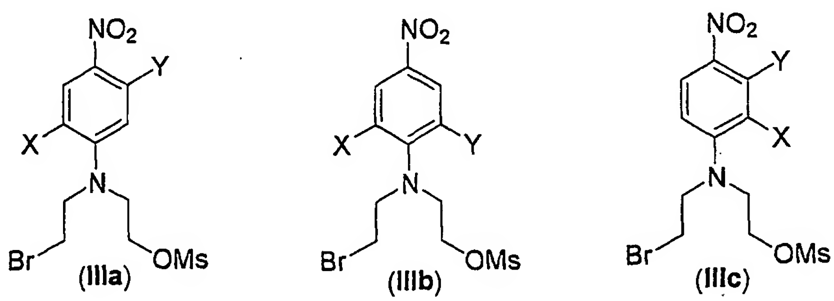
the method including the step of

(i) reacting a compound of



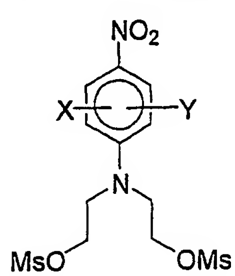
with an amount of an alkali metal halide or mesylate halide in a polar solvent to give a unsymmetrical halo-mesylate compound.

In a more preferred embodiment the method of preparing a nitroaniline-based unsymmetrical mustard represented by one of formulae (**IIIa-IIIc**)



wherein X, Y, are as defined above for a compound of Formula (I); and pharmaceutically acceptable derivatives and salts thereof; the method including the step of

(ii) reacting a compound of



with an amount of LiBr in a polar solvent to give a bromo mesylate of one of formulae (**IIIa-IIIc**).

It is preferred in the methods defined above that the polar solvent is selected from acetonitrile, dimethylformamide, ethyl acetate, triethylamine, acetone and mixtures thereof.

It is preferred in the methods defined above that the alkali metal halide is selected from one or more of the following; LiCl, LiBr, NaI and NaBr.

In a third aspect there is provided a compound of formula (I) obtained by any one of the preparative methods defined above.

In a fourth aspect, the present invention provides a method for the use as prodrugs suitable for GDEPT (gene-dependent enzyme-prodrug therapy) in conjunction with at least one nitroreductase enzyme, as hypoxia-selective cytotoxins, including the step of administering a compound of Formula I as defined above or a compound of Formulae Ia-Ic, IIa – IIc and IIIa-c as defined above or a mixture thereof in a "therapeutically effective amount" to tumour cells in a subject.

Preferably, the nitroreductase enzyme is encoded for by the nfsB gene of either *E.Coli* or by *Clostridia* species.

In a fifth aspect, the present invention provides a method for the use as prodrugs suitable for GDEPT (gene-dependent enzyme-prodrug therapy) in conjunction with at least one nitroreductase enzyme, as an anticancer agent including the step of administering a compound of Formula I as defined above or a compound of Formulae Ia-Ic, IIa – IIc and IIIa-c as defined above or a mixture thereof in a "therapeutically effective amount" to target tumour cells in a subject.

Preferably the nitroreductase enzyme is encoded for by the nfsB gene of either *E.Coli* or by *Clostridia* species.

In a sixth aspect of the present invention, there is provided a method of cell ablation therapy utilising at least one nitroreductase enzyme, wherein the method includes the step of administering a compound of Formula I as defined above or a compound of Formulae

Ia-Ic, IIa – IIc and IIIa-c as defined above or a mixture thereof in a "therapeutically effective amount" to ablate tumour cells in tissue in a subject, wherein said tissue expresses the at least one nitroreductase enzyme.

Preferably the nitroreductase enzyme is encoded for by the nfsB gene of either *E.Coli* or by *Clostridia* species.

Preferably, the cell ablation therapy provides a substantially minimal bystander effect.

In a seventh aspect of the present invention there is provided a pharmaceutical composition including a therapeutically effective amount of a compound of formula I or a compound of formulae Ia-c, IIa-c, IIIa-c or a mixture thereof, and a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser.

The pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser should preferably be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, such as cutaneous, subcutaneous, or intravenous. It is to be appreciated that these factors could be readily determined by someone skilled in the art without undue experimentation.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier or an adjuvent. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included. A capsule may comprise a solid carrier such as gelatin.

For intravenous, cutaneous or subcutaneous injection, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has a suitable pH, isotonicity and stability. Those of relevent skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium

Chloride injection, Ringer's injection, Lactated Ringer's injection. Preservatives, stabilisers, buffers antioxidants and/or other additives may be included as required.

In an eighth aspect of the present invention there is provided, the use in the manufacture of a medicament of an effective amount of a compound of Formula I as defined above or a compound of Formulae Ia-Ic, IIa – IIc and IIIa-c as defined above, for use in GDEPT to target cancer cells in a subject in need thereof.

In a ninth aspect of the present invention there is provided, the use in the manufacture of a medicament of an effective amount of a compound of Formula I as defined above or a compound of Formulae Ia-Ic, IIa – IIc and IIIa-c as defined above, for use in cell ablation therapy to target cancer cells in a subject in need thereof.

While the compounds of the present invention will typically be used to target tumour cells or tumour tissues in human subjects, they may be used to target tumour cells or tissues in other warm blooded animal subjects such as other primates, farm animals such as cattle, and sports animals and pets such as horses, dogs, and cats.

As used throughout the specification the term "therapeutically effective amount", is to be understood as an amount of a compound of Formula I as defined above or a compound of any one of compounds Ia-c, IIa-c and IIIa-c as defined above or a mixture thereof that is sufficient to show benefit to a subject with cancer cells. The actual amount, rate and time-course of administration, will depend on the nature and severity of the disease being treated. Prescription of treatment is within the responsibility of general practitioners and other medical doctors.

It is to be understood that the compounds of the invention as defined above may be administered alone or in combination with other treatments, especially radiotherapy, either simultaneously or sequentially dependent upon the condition to be treated.

As used throughout the specification the pharmaceutically acceptable derivatives and salts thereof include acid derived salts formed from are hydrochloric, sulfuric, phosphoric, acetic, citric, oxalic, malonic, salicylic, malic, fumaric, succinic, ascorbic, maleic,

methanesulfonic, isethionic acids and the like and base derived salts formed from sodium and potassium carbonate, sodium and potassium hydroxide, ammonia, triethylamine, triethanolamine and the like.

The technique of cell ablation therapy, would be known to someone skilled in the art. This therapy can be used to selectively ablate specified target cells or tissue through specific enzymatic expression of a nitroreductase for example, that is specifically expressed by the tissue and which can then be employed to active a prodrug into an active metabolite to ablate the specified target cells or tissue. (Gusterson *et al. Endocrine Related Cancer*, 1997, 4, 67-74.)

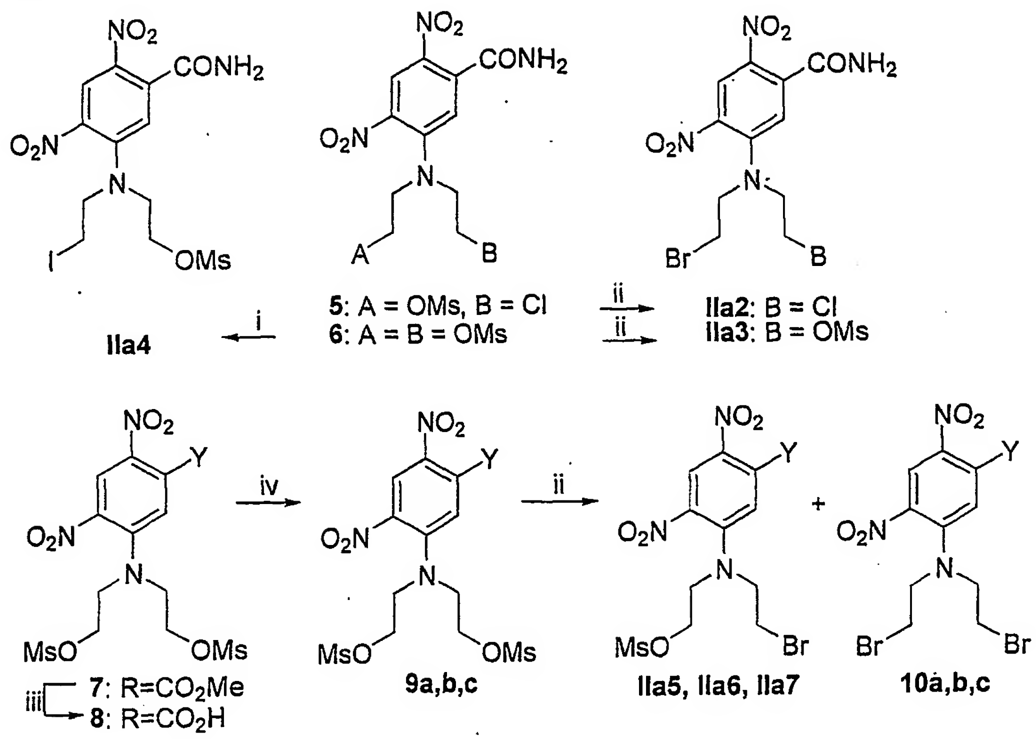
The expression "substantially minimal bystander effect" is to be understood as meaning that the killing of adjoining non-targeted tumour cells is minimal as a result of diffusion between the targeted tumour cells and non-targeted tumour cells of an activated metabolite that arises from the enzymatic activation of a compound of Formula I as defined above or a compound of any one of compounds Ia-c, IIa-c and IIIa-c as defined above or a mixture thereof.

Further aspects of the present invention will become apparent from the following description given by way of example only and with reference to the accompanying synthetic schemes.

DETAILED DESCRIPTION OF THE INVENTION

The compounds of formula (I) and the acid addition salts and N-oxides thereof may be prepared by the processes outlined in Schemes 1-3, examples of which are found in Examples A-C.

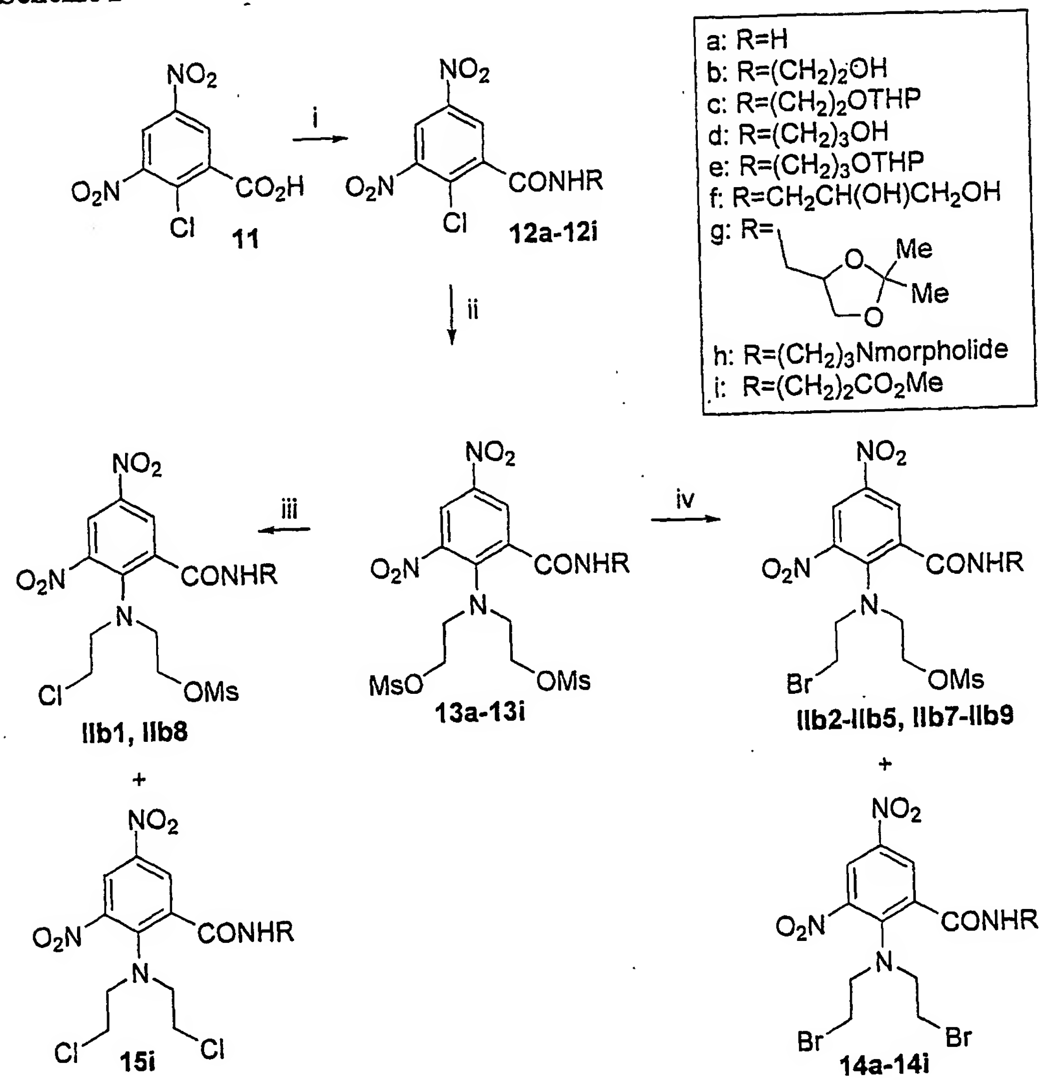
Scheme 1.



Reagents:
(i) NaI/DMF;
(ii) LiBr/MeCN;
(iii) KOH;
(iv) $SOCl_2$, then RNH_2.

a: $Y=(CH_2)_2OH$
b: $Y=(CH_2)_3OH$
c: $Y=CH_2CH(OH)CH_2OH$

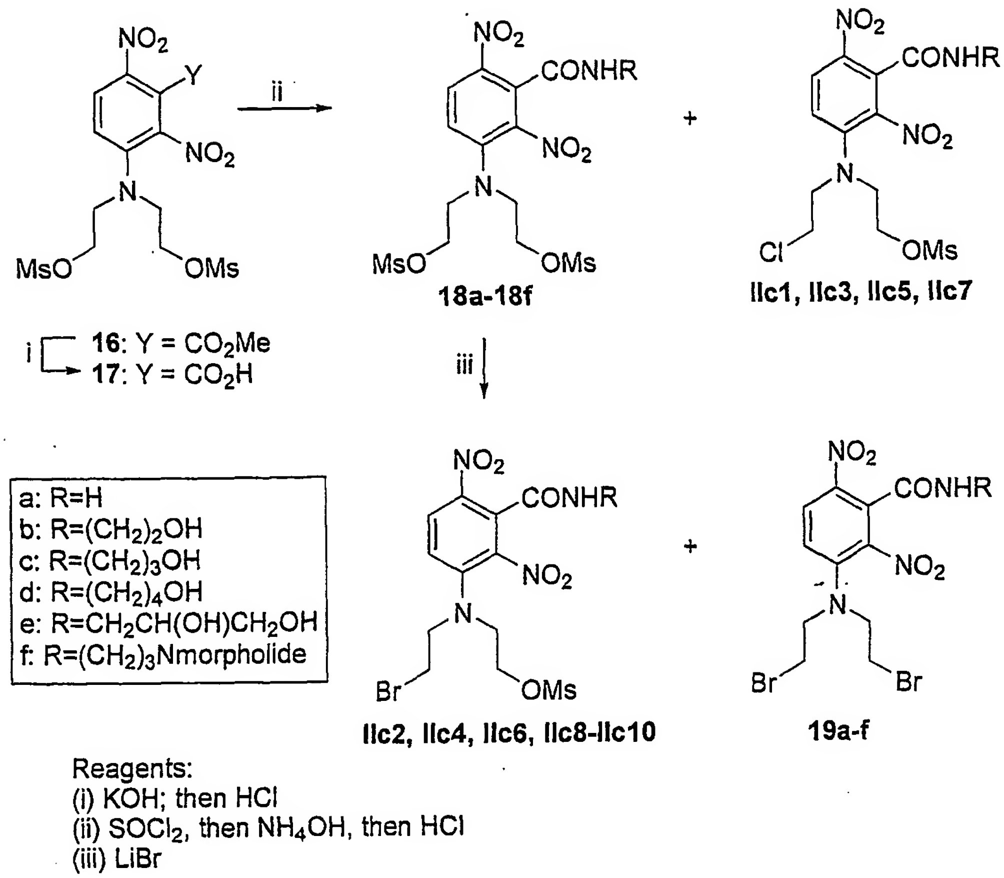
Scheme 2



Reagents:

(i) RNH_2;
(ii) $HN(CH_2CH_2OH)_2$, then MsCl;
(iii) LiCl;
(iv) LiBr.

Scheme 3.

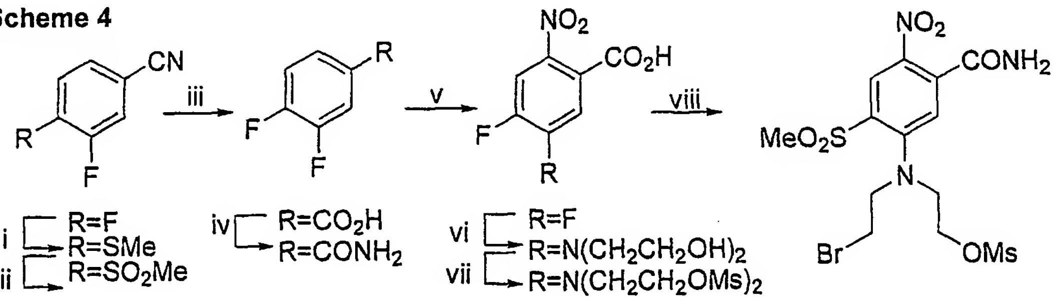


In Schemes 1-3, the key reaction is reaction of the dimesylates **6**, **9**, **13a-13g** and **18a-18d** with strictly controlled amounts of LiBr or NaI in a polar solvent like DMF or MeCN to give the unsymmetrical bromo- and iodo-mesylate mustards. The method can also be adapted to reaction of the known chloromesylate (**5**) to give the unsymmetrical chloro/bromo mustard **IIa2**. While this reaction gives varying amounts of the corresponding bis(bromo) or bis(iodo) compounds as well, these can be easily separated by chromatography to give the pure unsymmetrical mustards.

Compounds of formula I wherein X represents SO_2Me, Y represents $CONR^2R^3$, and A and B each independently represent halogen or OSO_2R^4 (with the proviso that $A \neq B$) can be prepared by the general route outlined (for a specific example) in Scheme 4, from 3,4-difluorobenzonitrile [after Atwell et al., *Anti-Cancer Drug Design*, **1996**, *11*, 553-567]. Reaction of this with NaSMe followed by oxidation provides the SO_2Me

group, and the nitrile is then elaborated to the $CONR^2R^3$ function. Displacement of the 4-F group with diethanolamine, followed by elaboration, gives the required asymmetric mustards.

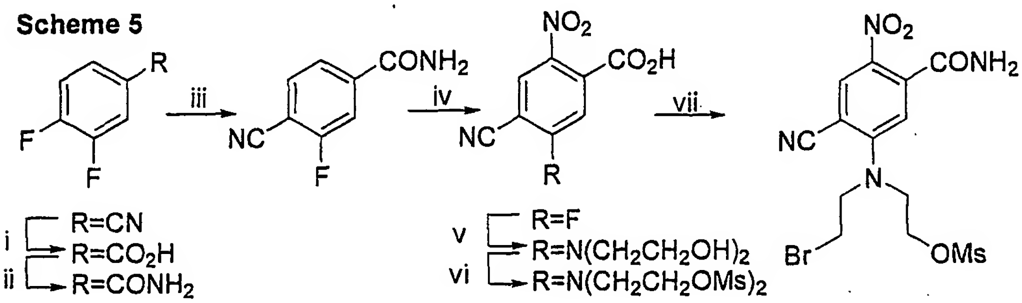
Scheme 4



(i) NaSMe; (ii) $NaBO_3$; (iii) H_2SO_4/AcOH; (iv) $SOCl_2$, then NH_4OH; (v) HNO_3/H_2SO_4; (vi) $HN(CH_2CH_2OH)_2$/DMSO; (vii) MsCl; (viii) LiBr/DMF.

Compounds of formula I wherein X represents CN, Y represents $CONR^2R^3$, and A and B each independently represent halogen or OSO_2R^4 (with the proviso that $A \neq B$) can be prepared by the general route outlined (as shown for a specific example) in Scheme 5, from 3,4-difluorobenzonitrile [after Atwell et al., *Anti-Cancer Drug Design*, **1996**, *11*, 553-567]. Conversion of the nitrile to a carboxamide (hydrolysis followed by amination), then displacement of the 3-F with TMS-CN, followed by reaction of the 4-F group with diethanolamine and subsequent elaboration as in Scheme 4 gives the required asymmetric mustard.

Scheme 5

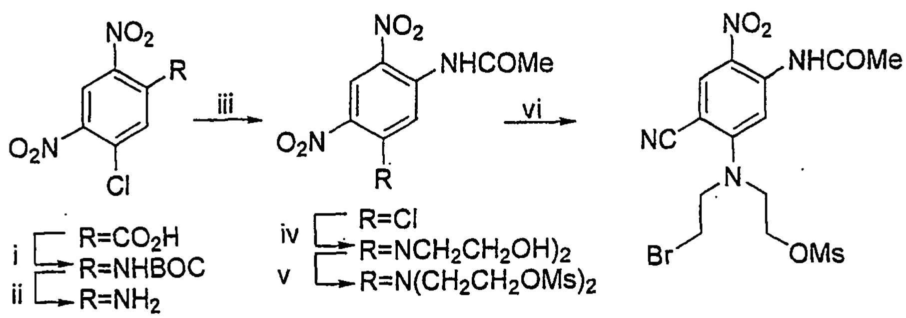


ii) H_2SO_4/AcOH; (ii) $SOCl_2$, then NH_4OH; (iii) TMSCN; (iv) HNO_3/H_2SO_4; (v) $HN(CH_2CH_2OH)_2$/DMSO; (vi) MsCl; (vii) LiBr/DMF.

Compounds of formula I wherein X represents NO_2, Y represents $NHCOR^2$, and A and B each independently represent halogen or OSO_2R^4 (with the proviso that $A \neq B$) can be prepared by the general route outlined (as shown for a specific example) in Scheme

6, from 2,4-dinitro-5-chlorobenzoic acid. Curtius reaction with DPPA, followed by hydrolysis and acetylation gives the acetamide. Reaction of the 5-Cl group with diethanolamine and subsequent elaboration as in Scheme 4 gives the required asymmetric mustard.

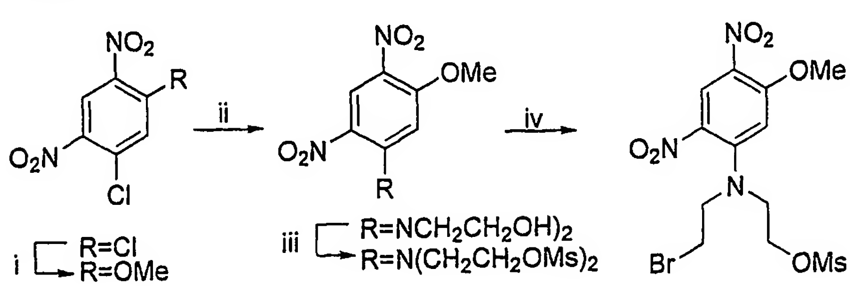
Scheme 6



ii) DPPA/t-BuOH; (ii) TFA; (iii) Ac_2O/pyridine; (iv) $HN(CH_2CH_2OH)_2$/DMSO; (v) MsCl; (vi) LiBr/DMF

Compounds of formula I wherein X represents NO_2, Y represents OR^2, and A and B each independently represent halogen or OSO_2R^4 (with the proviso that $A \neq B$) can be prepared by the general route outlined (as shown for a specific example) in Scheme 7, from 1,5-dichloro-2,4-dinitrobenzene. Reaction of the more active 1-Cl group with NaOMe gives the methyl ether, and subsequent elaboration of the 5-Cl group as in Scheme 4 gives the required asymmetric mustard.

Scheme 7

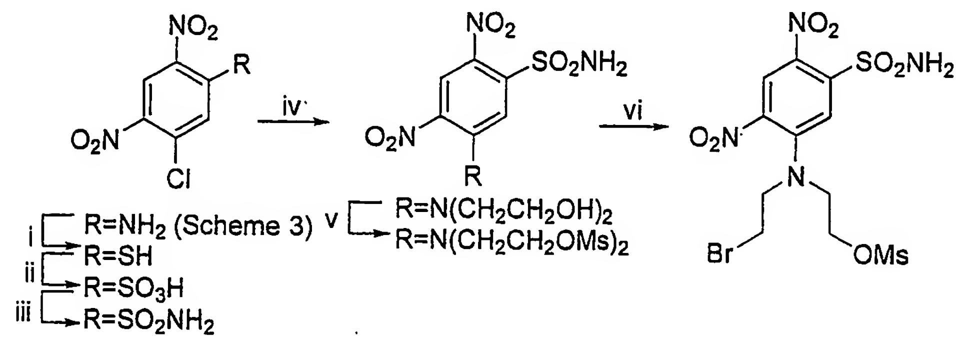


ii) Na/MeOH; (ii) $HN(CH_2CH_2OH)_2$/DMSO; (iii) MsCl; (iv) LiBr/DMF

Compounds of formula I wherein X represents NO_2, Y represents SO_2NHR^2, and A and B each independently represent halogen or OSO_2R^4 (with the proviso that $A \neq B$) can be prepared by the general route outlined (as shown for a specific example) in Scheme

8, from 5-chloro-2,4-dinitroaniline (see Scheme 6). Diazotization followed by oxidation and amination provides the sulphonamide [Herbert RB & Hollman RG. *Tetrahedron* **1965**, *21*, 663-675], and subsequent elaboration of the 5-Cl group as in Scheme 4 gives the required asymmetric mustard.

Scheme 8



(i) diazotize, then H_2S; (ii) oxone; (iii) $SOCl_2$, then NH_4OH;(iv) $HN(CH_2CH_2OH)_2$/dioxane; (V) MsCl; (vi) LiBr/DMF

The following Table 1 sets out physicochemical data for 25 compounds within the general formula I, representative of it, and preparable by the processes of the invention.

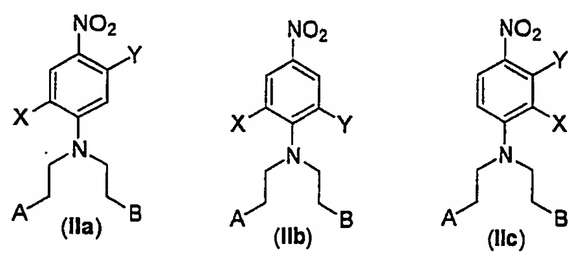


Table 1

No	Y	X	A	B	PRIOR ART COMPOUND		
5	$CONH_2$	NO_2	Cl	OMs	[Friedlos et al., *J. Med Chem.* 1997, 40, 1270]		
Examples of formula IIa							
No	Y	X	A	B	mp (°C)	formula	analyses
IIa2	$CONH_2$	NO_2	Cl	Br	153	$C_{11}H_{12}BrClN_4O_5$	C,H,N,Cl
IIa3	$CONH_2$	NO_2	Br	OMs	160-161	$C_{12}H_{15}BrN_4O_8S$	C,H,N,Br
IIa4	$CONH_2$	NO_2	I	OMs	160	$C_{12}H_{15}IN_4O_8S$	C,H,N,I

IIa5	CONH(CH2)2OH	NO_2	Br	OMs	102-104	$C_{14}H_{19}Br_4N_4O_9S$	C,H,N,Br
IIa6	CONH(CH2)3OH	NO_2	Br	OMs	gum	$C_{15}H_{21}Br_4N_4O_9S$	HRMS
IIa7	CONHCH2CH(OH)-CH2OH	NO_2	Br	OMs	117-118	$C_{15}H_{21}BrN_4O_{10}S$	C,H,N,Cl

*Examples of formula **IIb***

No	Y	X	A	B	mp (°C)	formula	analyses
IIb1	$CONH_2$	NO_2	Cl	OMs	155-157	$C_{12}H_{15}ClN_4O_8S$	C,H,N,Cl
IIb2	$CONH_2$	NO_2	Br	OMs	153-154	$C_{12}H_{15}BrN_4O_8S$	C,H,N,Br
IIb3	$CONH(CH_2)_2OH$	NO_2	Br	OMs	gum	$C_{14}H_{19}BrN_4O_9S$	HRMS
IIb4	$CONH(CH_2)_2OH$	NO_2	I	OMs	gum	$C_{14}H_{19}IN_4O_9S$	
IIb5	$CONH(CH_2)_3OH$	NO_2	Br	OMs	oil	$C_{15}H_{21}BrN_4O_9S$	
IIb6	$CONHCH_2CH(OH)$-CH_2OH	NO_2	Br	OMs	gum	$C_{15}H_{21}BrN_4O_{10}S$	C,H,N,Br
IIb7	$CONH(CH_2)_3$Nmorph	NO_2	Br	OMs	gum	$C_{19}H_{28}BrN_5O_9S$	HRMS
IIb8	$CONH(CH_2)_2CO_2Me$	NO_2	Cl	OMs	oil	$C_{16}H_{21}ClN_4O_{10}S$	HRMS
IIb9	$CONH(CH_2)_2CO_2Me$	NO_2	Br	OMs	gum	$C_{16}H_{21}BrN_4O_{10}S$	HRMS

*Examples of formula **IIc***

No	Y	X	A	B	mp (°C)	formula	analyses
IIc1	$CONH_2$	NO_2	Cl	OMs	134-136	$C_{12}H_{15}ClN_4O_8S$	C,H,N,S
IIc2	$CONH_2$	NO_2	Br	OMs	143-145	$C_{12}H_{15}BrN_4O_8S$	C,H,N,Br
IIc3	$CONH(CH_2)_2OH$	NO_2	Br	OMs	94-97	$C_{14}H_{19}BrN_4O_9S$	C,H,N
IIc4	$CONH(CH_2)_3OH$	NO_2	Cl	OMs	104-109	$C_{15}H_{21}ClN_4O_9S$	C,H,N,Cl
IIc5	$CONH(CH_2)_3OH$	NO_2	Br	OMs	115-117	$C_{15}H_{21}BrN_4O_9S$	C,H,N
IIc6	$CONH(CH_2)_4OH$	NO_2	Br	OMs	114-117	$C_{16}H_{23}BrN_4O_9S$	C,H,N
IIc7	$CONHCH_2CH(OH)$-CH_2OH	NO_2	Cl	OMs	100-105	$C_{15}H_{21}ClN_4O_{10}S$	C,H,N,Cl
IIc8	$CONH_2CH_2CH(OH)CH_2OH$	NO_2	Br	OMs	108-110	$C_{15}H_{21}BrN_4O_{10}S$	C,H,N,Br
IIc9	$CONH(CH_2)_3$Nmorph	NO_2	Cl	OMs	113-116	$C_{19}H_{28}ClN_5O_9S$	HRMS
IIc10	$CONH(CH_2)_3$Nmorph	NO_2	Br	OMs	114-117	$C_{19}H_{28}BrN_5O_9S$	HRMS

The following Examples A-C illustrate the preparation of compounds representative of the general formula (I).

Example A : Preparation of analogues of class IIa by the method outlined in Scheme 1.

5-[(2-Bromoethyl)(2-chloroethyl)amino]-2,4-dinitrobenzamide (IIa2). A mixture of 2-[5-(aminocarbonyl)(2-chloroethyl)-2,4-dinitroanilino]ethyl methanesulfonate (**5**) [Friedlos et al., *J. Med. Chem.* 1997, 40, 1270] (0.91 g, 2.2 mmol) and LiBr (0.21 g, 2.4 mmol) in anhydrous MeCN (25 mL) was stirred under reflux for 1.5 h, then concentrated under reduced pressure. The residue was chromatographed on silica gel, eluting with CH_2Cl_2/EtOAc (3:2) to give a crude product contaminated with the corresponding dibromo mustard. Purification by multiple recrystallisations from EtOAc/I-Pr_2O gave **IIa2** (595 mg, 68%): mp 153 °C; ^1H NMR [$(CD_3)_2SO$] δ 8.52 (s, 1 H, H-3), 8.17 & 7.82 (2 x s, 2 H, $CONH_2$), 7.43 (s, 1 H, H-6), 3.82 (t, J = 5.8 Hz, 2 H, CH_2Cl), 3.77-3.63 (m, 6 H, N(CH_2-)CH_2CH_2Br). Anal. Calc for $C_{11}H_{12}BrClN_4O_5$: C, 33.4; H, 3.1; N, 14.2; Cl, 9.6. Found: C, 33,4; H, 3.0; N, 14.1; Cl, 8.9%.

2-[5-(Aminocarbonyl)(2-bromoethyl)-2,4-dinitroanilino]ethyl methanesulfonate (IIa3). A mixture of 2-(5-(aminocarbonyl){2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilino)ethyl methanesulfonate (**6**) [Friedlos et al., *J. Med Chem.*, 1997, 40, 1270] (1.60 g, 3.4 mmol) and LiBr (356 mg, 4.1 mmol) in anhydrous MeCN (30 mL) was stirred under reflux for 1 h. The mixture was concentrated under reduced pressure and the residue was chromatographed on silica gel. Elution with EtOAc/CH_2Cl_2 (11:9) gave the dibromo mustard, while further elution with EtOAc/CH_2Cl_2 (3:1) gave **IIa3** (0.61 g, 39%): mp (EtOAc/I-Pr_2O) 160-161°C; ^1H NMR [$(CD_3)_2SO$] δ 8.53 (s, 1 H, H-3), 8.14 &7.83 (2 x s, 2 H, $CONH_2$), 7.46 (s, 1 H, H-6), 4.33 (t, J = 5.1 Hz, 2 H, CH_2O), 3.74 (t, J = 5.1 Hz, 2 H, CH_2CH_2O), 3.70 (br s, 4 H, CH_2CH_2Br), 3.14 (s, 3 H, CH_3). Anal. Calcd for $C_{12}H_{15}BrN_4O_8S$: C, 31.7; H, 3.3; N, 12.3; Br, 17.6. Found: C, 32.0; H, 3.4; N, 12.2; Br, 17.7%.

2-[5-(Aminocarbonyl)(2-iodoethyl)-2,4-dinitroanilino]ethyl methanesulfonate (IIa4). A mixture of **6** (1.12 g, 2.38 mmol) and NaI (0.46 g, 3.07 mmol) in anhydrous MeCN (20 mL) was stirred at reflux for 1 h. The mixture was concentrated under reduced pressure and the residue was chromatographed on silica gel. Elution with EtOAc/CH_2Cl_2 (1:1)

gave the diiodo mustard, while further elution with $EtOAc/CH_2Cl_2$ (3:1) gave **IIa4** (0.49 g, 41%): mp ($Me_2CO/EtOAc/I\text{-}Pr_2O$) 160 °C; ^1H NMR [$(CD_3)_2SO$] δ 8.52 (s, 1 H, H-3), 8.14 & 7.83 (2 x s, 2 H, NH_2), 7.44 (s, 1 H, H-6), 4.33 (t, J = 5.1 Hz, 2 H, CH_2O), 3.73 (t, J = 5.1 Hz, 2 H, CH_2CH_2O), 3.65 (t, J = 6.9 Hz, 2 H, CH_2CH_2I), 3.40 (t, J = 6.9 Hz, 2 H, CH_2I), 3.13 (s, 3 H, CH_3). Anal. Calcd for $C_{12}H_{15}IN_4O_8S$: C, 28.7; H, 3.0; N, 11.2; I, 25.3. Found: C, 29.4; H, 3.0; N, 11.0; I, 25.0%.

2-((2-Bromoethyl)5-{[(2-hydroxyethyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate (IIa5). A stirred solution of methyl 5-[bis(2-hydroxyethyl)amino]-2,4-dinitrobenzoate [Palmer et al., *J. Med. Chem* 1994, 37, 2175] (5.50 g, 16.7 mmol) and Et_3N (5.82 mL, 41.8 mmol) in dry CH_2Cl_2 (50 mL) was treated dropwise at 0 °C with MsCl (3.14 mL, 40.0 mmol). After 30 min, 10% aqueous $KHCO_3$ (100 mL) was added, and the mixture was stirred for a further 30 min at 0 °C and then diluted with pet. ether (500 mL). The precipitated product was collected and washed with water and iPr_2O to give methyl 5-(bis{2-[(methylsulfonyl)oxy]ethyl}amino)-2,4-dinitrobenzoate (7) (7.44 g, 92%): mp (CH_2Cl_2/pet. ether) 157-158 °C; ^1H NMR [$(CD_3)_2SO$} δ 8.62 (s, 1 H, H-3), 7.77 (s, 1 H, H-6), 4.35 (t, J = 5.1 Hz, 4 H, $2xCH_2O$), 3.88 (s, 3 H, CO_2CH_3), 3.73 (t, J = 5.1 Hz, 4 H, $N(CH_2)CH_2$), 3.13 (s, 6 H, $2xSO_2CH_3$). Anal calcd for $C_{14}H_{19}N_2O_{12}S_2$: C, 34.6; H, 3.9; N, 8.7; S, 13.2. Found: C, 34.8; H, 3.7; N, 8.9; S, 13.1%.

Hydrolysis of 7 (3.0 g, 6.18 mmol) with 3 N KOH (40 mL) in dioxane (200 mL) at room temperature for 15 min followed by acidification with 1 N HCl and extraction with EtOAc gave a quantitative yield of 5-(bis{2-[(methylsulfonyl)oxy]ethyl}amino)-2,4-dinitrobenzoic acid (**8**), mp 200-210 °C, which was used for the next step without further purification; ^1H NMR [$(CD_3)_2SO$] δ 14.1 (v br s, 1 H, CO_2H), 8.57 (s, 1 H, H-3), 7.69 (s, 1 H, H-6), 4.34 (t, J = 5.1 Hz, 4 H, $2xCH_2O$), 3.72 (t, J = 5.1 Hz, 4 H, $2xCH_2CH_2O$), 3.13 (s, 6 H, $2xCH_3$).

A suspension of **8** (3.20 g, 6.79 mmol) in $SOCl_2$ (60 mL) containing DMF (2 drops) was heated under reflux for 1 h. Evaporation of the solvent under reduced pressure, followed by azeotroping in with benzene gave the crude acid chloride, which was dissolved in dry Me_2CO (80 mL) and treated at 0 °C with 2-aminoethanol (1.24 g, 20.3 mmol). After stirring at 0 °C for 5 min, the mixture was acidified to pH 2-3 with 0.2 N HCl, concentrated to half volume, and then solid NaBr was added. The mixture was extracted with EtOAc (2x) and the combined extracts were washed with saturated NaBr

solution, dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel, eluting with EtOAc/MeOH (15:1) to give 2-(5-{[(2-hydroxyethyl)amino]carbonyl}{2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilino)ethyl methanesulfonate (**9a**) (2.87g , 82%) as a gum that was used directly.

A mixture of **9a** (1.80 g, 3.50 mmol) and LiBr (0.43 g, 4.95 mmol) in DMF (5 mL) was stirred at 60 °C for 2 h. The reaction was then poured into saturated NaBr solution and extracted with EtOAc (2x). The combined extracts were washed with saturated NaBr solution, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was chromatographed on silica gel, eluting with EtOAc, to give 5-[bis(2-bromoethyl)amino]-*N*-(2-hydroxyethyl)-2,4-dinitrobenzamide (**10a**) (0.78 g, 46%): mp (MeOH/EtOAc/pet. ether) 151-152 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.73 (t, J = 5.7 Hz, 1 H, CONH), 8.53 (s, 1 H, H-3), 7.43 (s, 1 H, H-6), 4.76 (t, J = 5.6 Hz, 1 H, OH), 3.77-3.64 (m, 8 H, $N(CH_2CH_2Br)_2$), 3.53 (q, J = 6.0 Hz, 2 H, CH_2OH), 3.31 (q, partially obscured, J = 6.1 Hz, 2 H, CONHCH_2). Anal. calcd for $C_{13}H_{16}Br_2N_4O_6$): C, 32.3; H, 3.3; 11.6; 33.0. Found: C, 32.6; H, 3.3; N, 11.6; Br, 33.3%.
Further elution with EtOAc/MeOH (9:1) gave **IIa5** (0.73 g, 42%): mp (EtOAc) 102-104 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.70 (t, J = 5.7 Hz, 1 H, CONH), 8.54 (s, 1 H, H-3), 7.46 (s, 1 H, H-6), 4.76 (J = 5.5 Hz, 1 H, OH), 4.34 (t, J = 5.1 Hz, 2 H, CH_2OSO_2), 3.74 (t, J = 5.1 Hz, 2 H, $CH_2CH_2OSO_2$), 3.70 (br s, 4 H, CH_2CH_2Br), 3.53 (q, J = 6.0 Hz, 2 H, CH_2OH), 3.31 (q, partially obscured, J = 6.1 Hz, 2 H, $CONHCH_2$), 3.14 (s, 3 H, CH_3). Anal. calcd for $C_{14}H_{19}BrN_4O_9S$: C, 34.3; H, 3.9; N, 11.0; Br, 15.9. Found: C, 33.8; H, 3.8; H, 11.2; Br, 16.0%.

2-((2-Bromoethyl)5-{[(3-hydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate (IIa6). 5-(Bis{2-[(methylsulfonyl)oxy]ethyl}amino)-2,4-dinitrobenzoic acid (**8**) was heated under reflux in excess $SOCl_2$ (60 mL) and catalytic DMF for 1 h. Evaporation under reduced pressure, followed by azeotroping in benzene, gave the crude acid chloride. This was dissolved in dry Me_2CO and treated at 0 °C with 3-amino-1-propanol at 0 °C for 5 min. The mixture was acidified to pH 2-3 with 0.2 N HCl, concentrated to half volume, and then solid NaBr was added, followed by extraction with EtOAc (2x). Evaporation, and chromatography of the residue on silica gel, eluting with EtOAc/MeOH (9:1), gave 2-(5-{[(3-

hydroxypropyl)amino]carbonyl}{2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilino)ethyl methanesulfonate (**9b**) (68%) as a yellow gum; ^{1}H NMR [$(CD_3)_2SO$] δ 8.54 (t, J = 5.7 Hz, 1 H), 8.53 (s, 1 h), 7.45 (s, 1 H), 4.43 (t, J = 5.1 Hz, 1 H), 4.33 (t, J = 5.2 Hz, 4 H), 3.69 (t, J = 5.2 Hz, 4 H), 3.5 7(q, J = 5.9 Hz, 2 H), 3.26 (after D_2O exchange, t, J = 7.0 Hz, 1 H), 3.12 (s, 6 H), 1,66 (pent, J = 6.7 Hz, 2 H). HRMS (FAB) calcd. for $C_{16}H_{25}N_4O_{12}S$ (MH^+) m/z 529.0910; found 529.0904.

A solution of **9b** in DMF was treated with LiBr (1.4 equiv.), and worked up as above, and the product was chromatographed on silica gel. Elution with EtOAc gave a small amount of the dibromo mustard **10b**, while elution with EtOAc/MeOH (19:1) gave **IIa6** (31%) as a yellow gum: ^{1}H NMR [$(CD_3)_2SO$] δ 8.60 (t, J = 5.6 Hz, 1 H), 8.54 (s, 1 H), 7.44 (s, 1 H), 4.45 (t, J = 5.2 Hz, 1 H), 4.33 (t, J = 5.1 Hz, 2 H), 3.74 (t, J = 5.2 Hz, 2 H), 3.72-3.66 (m, 4 H), 3.49 (q, J = 5.9 Hz, 2 H), 3.27 (after D_2O exchange, t, J = 7.0 Hz, 2 H), 3.14 (s, 3 H), 1.68 (pent, J = 6.7 Hz, 2 H). HRMS (FAB) calcd. for $C_{15}H_{22}{}^{79}BrN_4O_9S$ (MH^+) m/z 515.0270; found 515.0283.

2-((2-Bromoethyl)-5-{[(2,3-dihydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate (IIa7). Reaction of the crude acid chloride made as above from acid **8** (2.9 g, 6.15 mmol) was dissolved in Me_2CO (100 mL), cooled in an ice-bath and treated with an excess of 3-amino-1,2-propanediol. After stirring for 10 min. the reaction mixture was acidified to pH 2-3 with 1 N HCl, most of the solvent was evaporated, and the residue was partitioned between water and EtOAc. The aqueous layer was re-extracted with EtOAc and the combined organic phases were dried and evaporated. The residue was adsorbed directly onto silica gel and chromatographed, elution with EtOAc/MeOH (from 50:1 to 10:1) giving 2-(5-{[(2,3-dihydroxypropyl)amino]carbonyl}{2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilino)ethyl methanesulfonate (**9c**) (2.92 g, 87%) as a yellow oil; ^{1}H NMR [$(CD_3)_2SO$] δ 8.66 (t, J = 5.8 Hz, 1 H, CONH), 8.54 (s, 1 H, H-3), 7.48 (s, 1 H, H-6), 4.81 (d, J = 5.0 Hz, 1 H, CHO*H*), 4.59 (t, J = 5.1 Hz, 1 H, CH_2OH), 4.35 (m, 4 H, 2x CH_2OMs), 3.66 (m, 4 H), 3.62 (m, 1 H), 3.46 – 3.36 (m, 4 H), 3.13 (s, 6 H); ^{13}C NMR δ 164.48, 147.09, 138.26, 137.27, 136.60, 124.17, 121.72, 70.02, 66.69, 63.68, 50.21, 42.68, 36.55. HRMS m/z $(M+1)^+$ required for $C_{16}H_{25}N_4O_{13}S_2$ 545.08596; Found 545.0856.

A solution of **9c** (1.28 g, 2.53 mmol) was dissolved in EtOAc (100 mL) and treated with LiBr (347 mg, 4.0 mmol) at 60 °C for 2 h. Volatiles were removed under reduced pressure, and the residue was adsorbed directly onto silica gel and chromatographed. Elution with EtOAc/MeOH (from 1:0 to 10:2) gave 5-[bis(2-bromoethyl)amino]-*N*-(2,3-dihydroxypropyl)-2,4-dinitrobenzamide (**10c**) (0.4 g, 31%) as a foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.71 (t, *J* = 5.8 Hz, 1 H, CONH), 8.53 (s, 1 H, H-3), 7.43 (s, 1 H, H-6), 4.86 (d, *J* = 5.0 Hz, 1 H, CHO*H*), 4.59 (t, *J* = 5.8 Hz, 1 H, CH_2OH), 3.70 – 3.10 (m, 13 H); ^{13}C NMR δ 164.61, 146.65, 137.99, 137.35, 136.52, 124.25, 121.20, 70.05, 63.73, 52.44, 42.76, 30.33. HRMS m/z $(M+1)^+$ required for $C_{14}H_{19}{}^{79}Br_2N_4O_7$ 512.9621; Found 512.9596.

Further elution gave **IIa7** (0.62 g, 46%): mp (EtOAc) 117-118 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.68 (t, *J* = 5.8 Hz, 1 H, CONH), 8.53 (s, 1 H, H-3), 7.46 (s, 1 H, H-6), 4.82 (d, *J* = 5.0 Hz, 1 H, CHO*H*), 4.56 (t, *J* = 5.1, 1 H, CH_2OH), 4.32 (m, 2 H, CH_2OMs), 3.75-3.60 (m, 7 H), 3.46-3.36 (m, 4 H), 3.13 (s, 3 H); ^{13}C NMR δ 164.48, 146.84, 138.05, 137.29, 136.52, 124.18, 121.40, 70.01, 66.74, 63.68, 52.89, 49.56, 42.69, 36.55, 30.20. Anal. Calcd for $C_{15}H_{21}BrN_4O_{10}S$: C, 34.1; H, 4.0; N, 10.6; Br, 15.0. Found: C, 34.0; H, 4.0; N, 10.5; Br, 15.2%.

Further elution gave starting material (**9c**) (0.27 g, 20%).

Example B: Preparation of analogues of class IIb by the method outlined in Scheme 2.

2-[2-(Aminocarbonyl)(2-chloroethyl)-4,6-dinitroanilino]ethyl methanesulfonate (IIb1). A solution of 2-[bis(2-hydroxyethyl)amino]-3,5-dinitrobenzamide [Friedlos et al., *J. Med. Chem*, 1997, 40, 1270] (2.5 g, 8 mmol) in CH_2Cl_2 (200 mL) was cooled in an ice-bath and Et_3N (8 mL) and MsCl (4 mL) were added in one portion. After stirred for 10 min, satd. $NaHCO_3$ (100 mL) was added, and after a further 30 min the aqueous phase was extracted with CH_2Cl_2 (2x70 mL), the combined organic phase were dried, concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel. Elution with EtOAc/petroleum ether (1:1 to 1:0), gave **IIb1** (0.6 g, 18%): mp (EtOAc/petroleum ether) 155-157 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.74 (d, *J* = 2.7 Hz, 1 H, H-5), 8.34 (d, *J* = 2.7 Hz, 1 H, H-3), 8.19 (s, 1 H, CONH), 7.99 (s, 1 H, CONH), 4.29 (m, 2 H, CH_2OMs), 3.73 (m, 2 H, CH_2Cl), 3.48 (m, 4 H,

2xCH$_2$N), 3.15 (s, 3 H, OSO$_2$CH$_3$); ^{13}C NMR δ 167.11, 145.98, 146.34, 140.84, 136.05, 127.26, 122.22, 67.49, 54.35, 51.34, 41.36, 36.46. Anal. Calcd for $C_{12}H_{15}ClN_4O_8S$: C, 35.1; H, 3.7; N, 13.7; Cl, 8.5. Found: C, 35.7; H, 3.9; N, 13.6; Cl, 8.7%. Further elution gave 2-(2-(aminocarbonyl){2-[(methylsulfonyl)oxy]ethyl}-4,6-dinitroanilino)ethyl methanesulfonate (**13a**) (3.0 g, 80%): mp (EtOAc) 149-150 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.73 (d, J = 2.8 Hz, 1 H, H-5), 8.35 (d, J = 2.9 Hz, 1 H, H-3), 8.19 (s, 1 H, CONH), 8.00 (s, 1 H, CONH), 4.31 (m, 4 H, 2x CH$_2$OMs), 3.49 (m, 4 H, 2x CH$_2$-N), 3.14 (s, 6 H, 2xOSO$_2$CH$_3$). Anal. Calcd for $C_{13}H_{18}N_4O_{11}S_2$: C, 33.2; H, 3.9; N, 11.9. Found: C, 33.7; H, 4.0; N, 11.8%.

2-[2-(Aminocarbonyl)(2-bromoethyl)-4,6-dinitroanilino]ethyl methanesulfonate (IIb2). A solution of dimesylate **13a** (1.62 g, 3.5 mmol) in warm EtOAc (100 mL) was treated with one portion of LiBr (400 mg, 4.7 mmol), and the mixture was heated to 60 °C for 2 h. Volatiles were removed under reduced pressure, and the residue was adsorbed directly onto silica gel and chromatographed. Elution with EtOAc/petroleum ether (1:1 to 1:0) gave the dibromide (0.31 g, 20%) as yellow solid. (lit., foam) [Friedlos et al., *J. Med. Chem.* 1997, 1270]. Further elution gave **IIb2** (0.85 g, 53%): mp (EtOAc/petroleum ether) 153-154 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.74 (d, J = 2.8 Hz, 1 H, H-5), 8.33 (d, J = 2.8 Hz, 1 H, H-3), 8.19 (s, 1 H, CONH), 7.99 (s, 1 H, CONH), 4.29 (m, 2 H, CH$_2$OMs), 3.60 (m, 2 H, CH$_2$Br), 3.49 (m, 4 H, 2xCH$_2$-N), 3.14 (s, 3 H, OSO$_2$CH$_3$); ^{13}C NMR δ 167.11, 145.75, 146.37, 140.92, 136.12, 127.24, 122.20, 67.53, 54.41, 51.16, 36.46, 29.73. Anal. Calcd for $C_{12}H_{15}BrN_4O_8S$: C, 31.7; H, 3.3; N, 12.3; Br, 17.4. Found: C, 31.4; H, 3.4; N, 12.3; Br, 17.8%.

2-((2-Bromoethyl)-2-{[(2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate (IIb3). 2-Aminoethanol (2.9 g, 47 mmol) in 5 mL of water was added in one portion to a solution of crude 2-chloro-3,5-dinitrobenzoic acid chloride [prepared from 2-chloro-3,5-dinitrobenzoic acid **11** (5.0 g, 18.3 mmol) with SOCl$_2$] in Me$_2$CO (50 mL) while cooling in an ice-bath. The mixture was stirred for 30 min, then acidified with 1N HCl to pH4-5 and concentrated under reduced pressure to remove the Me$_2$CO. EtOAc (100 mL) was added, and after 2h a white solid was collected, washed with EtOAc and air-dried to give 2-chloro-3,5-dinitro-*N*-(2-hydroxyethyl)benzamide (**12b**) (3.0 g, 36%): mp (EtOAc) 159-160 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.99 (d, J = 2.6

Hz, 1 H, H-5), 8.86 (m, 1 H, CONH), 8.56 (d, J = 2.6 Hz, 1 H, H-3), 4.83 (m, 1 H, -OH), 3.54 (m, 4 H) which was used for next step without further purification.

A solution of **12b** (0.6 g, 2.14 mmol) in CH_2Cl_2 was cooled in an ice-bath, and 3,4-dihydro-2*H*-pyran (2.0 mL) and p-toluenesulfonic acid (0.1 g) were added. The reaction mixture was stirred for 2 h, then concentrated under reduced pressure. Chromatography of the residue on silica gel, eluting with EtOAc/petroleum ether (from 1:2 to 2:1), gave 2-chloro-3,5-dinitro-*N*-[2-(tetrahydro-2*H*-pyran-2-yloxy)ethyl]benzamide (**12c**) (0.8 g, 100%): as an oil; ^{1}H NMR [$(CD_3)_2SO$] δ 8.67 (d, J = 2.6 Hz, 1 H, H-4), 8.60 (d, J = 2.6 Hz, 1 H, H-6), 7.02 (m, 1 H, CONH), 4.54 (m, 1 H), 4.00-3.50 (m, 6 H), 1.84-1.75 (m, 6 H) which was used for next step without further purification. Reaction of **12c** with diethanolamine, followed by MsCl/Et_3N as described above, gave 2-[{2-[(methylsulfonyl)oxy]ethyl}-4,6-dinitro-6-({[2-(tetrahydro-2*H*-pyran-2-yloxy)ethyl]amino}carbonyl)anilino]ethyl methanesulfonate (**13c**) (1.28 g, 100%): as a yellow foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.63 (d, J = 2.9 Hz, 1 H, H-5), 8.51 (d, J = 2.9 Hz, 1 H, H-3), 4.55 (m, 1 H), 4.39 (m, 4 H), 4.00-3.59 (m, 10 H), 3.15 (s, 3 H), 3.03 (s, 3 H), 1.64-1.39 (m, 6 H) which was used in the next step without further purification.

A solution of **13c** (1.28 g, 2.14 mmol) in THF (60 mL) was treated with 1 N HCl (40 mL), and the solution was stirred at 20 °C for 1 h, then diluted with water (100 mL), neutralized with satd. $NaHCO_3$, and extracted with EtOAc (3x80 mL). The combined organic phases were washed with brine and dried, the solvent was evaporated, and the residue was purified by chromatography on silica gel, eluting with EtOAc/MeOH(from 1:0 to 100:2), to give **13b** (0.84 g, 76%): as a yellow foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.78 (m, 1 H, CONH), 8.74 (d, J = 2.7 Hz, 1 H, H-5), 8.36 (d, J = 2.7 Hz, 1 H, H-3), 4.29 (m, 4 H, $2xCH_2OMs$), 3.56 (m, 2 H), 3.45 (m, 6 H), 3.14 (s, 6 H, $2xOSO_2CH_3$); ^{13}C NMR δ 165.37, 146.27, 145.06, 140,63, 135.78, 127.62, 122.32, 67.26, 59.17, 51.26, 42.14, 36.44.

Treatment of **13c** (0.49 g, 0.95 mmol) with LiBr (0.100 g, 1.2 mmol) in EtOAc (60 mL) at 60 °C for 3 h, and chromatography of the product on silica gel, eluting with EtOAc/petroleum ether (from 2:1 to 1:0) gave the dibromide (**14c**) (0.24 g, 53%). Further elution gave **IIb3** (0.20 g, 42%): as yellow foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.77 (m, 1 H, CONH), 8.74 (d, J = 2.7 Hz, 1 H, H-5), 8.36 (d, J = 2.7 Hz, 1 H, H-3), 4.28

(m, 2 H, CH_2OMs), 3.58 (m, 4 H), 3.44 (m, 4 H), 3.14 (s, 3 H, OSO_2CH_3); ^{13}C NMR δ 165.33, 145.79, 145.20, 140,87, 135.11, 127.50, 122.19, 67.49, 59.18, 54.21, 50.99, 42.09, 36.44, 29.68. HRMS m/z $(M+1)^+$ required for $C_{14}H_{20}{}^{79}BrN_4O_9S$ 499.01344; Found 499.01324.

2-((2-Iodoethyl)-2-{[(2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate (IIb4).Treatment of **13b** (6.7 g, 13.0 mmol) with NaI (2.9 g, 20 mmol) in EtOAc (200 mL) at 60 °C for 3 h, and chromatography of the product on silica gel, eluting with EtOAc/petroleum ether (from 2:1 to 1:0) gave 2-[bis(2-iodoethyl)amino]-*N*-(3-hydroxyethyl)-3,5-dinitrobenzamide (3.3 g, 44%) as a yellow solid: mp (EtOAc/petroleum ether) 129-131 °C; ^1H NMR $[(CD_3)_2SO]$ δ 8.72 (d, J = 2.8 Hz, 1 H, H-4), 8.70 (m, 1 H, CONH), 8.32 (d, J = 2.8 Hz, 1 H, H-6), 4.80 (m, 1 H), 3.55 (m, 2 H), 3.43 (m, 4 H), 3.31 (m, 6 H); ^{13}C NMR δ 165.3, 145.2, 144.7, 141.0, 136.3, 127.3, 122.0, 59.3, 54.7, 42.1, 2.94.

Later eluates gave **IIb4** (1.35 g, 19%) as a yellow foam; ^1H NMR $[(CD_3)_2SO]$ δ 8.74 (d, J= 2.8 Hz, 1H, H-4), 8.74 (m, 1H, CONH), 8.34 (d, J=2.8 Hz, 1 H, H-6), 4.28 (m, 2 H), 3.56 (m, 2 H), 3.43 (m, 2 H), 3.31 (m, 6 H), 3.13 (s, 3 H); ^{13}C NMR δ 165.3, 145.5, 145.2, 140.8, 136.1, 127.4, 122.1, 67.5, 59.2, 55.4, 50.6, 42.1, 36.5, 2.6. HRMS (FAB) Calcd. For $C_{14}H_{20}IN_4O_9S$ $[M+H^+]$ *m/z* 546.9996. Found; 546.9997.

2-((2-Bromoethyl)-2-{[(2-hydroxypropyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate (IIb5). A solution of **12d** (1.22 g, 4.0 mmol) in 50 mL of CH_2Cl_2 was cooled in an ice-bath, and 3,4-dihydro-2*H*-pyran (1.0 mL) and p-toluenesulfonic acid (0.1 g) were added. The reaction mixture was stirred for 2 h, then concentrated under reduced pressure. Chromatography of the residue on silica gel, eluting with EtOAc/petroleum ether (from 1:2 to 2:1), gave 2-chloro-3,5-dinitro-*N*-[2-(tetrahydro-2*H*-pyran-2-yloxy)propyl]benzamide (**12e**) (1.45 g, 94%): as a pale yellow oil; ^1H NMR $[(CD_3)_2SO]$ δ 8.99 (d, J = 2.7 Hz, 1 H, H-4), 8.81 (m, 1 H, CONH), 8.51 (d, J = 2.7 Hz, 1 H, H-6), 4.57 (m, 1 H), 3.72 (m, 2 H), 3.46-3.25 (m, 4 H), 1.82-1.44 (m, 8 H). ^{13}C NMR δ 162.7, 148.4, 145.9, 140.3, 128.2, 125.8, 120.5, 98.0, 64.2, 61.3, 36.5, 30.2, 28.9, 24.9, 19.1. HRMS (FAB) Calcd. For $C_{15}H_{19}{}^{35}ClIN_3O_7$ $[M+H^+]$ *m/z* 388.0912. Found; 388.0915.

Reaction of **12e** (1.45 g, 3.75 mmol) with diethanolamine (1.67 g) as above gave 2-[bis(2-hydroxyethyl)amino]-3,5-dinitro-*N*-[2-(tetrahydro-2*H*-pyran-2-yloxy)propyl]benzamide (1.62 g, 95%) as a yellow foam that was used directly; ^{1}H NMR [$(CD_3)_2SO$] δ 8.96 (m, 1H, CONH), 8.66 (d, J= 2.8 Hz, 1H, H-4), 8.31 (d, J=2.8 Hz, 1H, H-6), 4.95 (m, 2H), 4.56 (m, 1H), 3.79-3.16 (m, 14H), 1.80-1.45 (m, 8 H); ^{13}C NMR δ 166.2, 148.1, 143.6, 139.3, 133.8, 128.9, 123.8, 98.5, 64.8, 61.7, 58.5, 54.6, 37.3, 30.6, 29.2, 25.4, 19.6. HRMS (FAB) Calcd. For $C_{19}H_{29}N_4O_6$ [$M+H^+$] *m/z* 457.1935. Found; 457.1939.

Reaction of the above diol (1.62 g, 3.55 mmol) with MsCl (2 mL) as above gave 2-[{2-[(methylsulfonyl)oxy]ethyl}-4,6-dinitro-6-({[2-(tetrahydro-2*H*-pyran-2-yloxy)propyl]-amino}carbonyl)anilino]ethyl methanesulfonate (**13e**) (2.17 g, 100%): as a yellow foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.71 (d, J = 2.8 Hz, 1H), 8.71 (m, 1H), 8.31 (d, J= 2.8 Hz, 1H), 4.26 (m, 4 H), 3.71-3.37 (m, 10 H), 3.13 (s, 6 H), 3.10 (m, 2 H), 1.82-1.43 (m, 8 H); ^{13}C NMR δ 165.1, 146.3, 145.4, 140.9, 135.9, 127.4, 122.2, 98.0, 67.2, 64.3, 51.4, 45.7, 36.5, 30.2, 28.7, 24.9, 19.1, 8.5. HRMS (FAB) Calcd. For $C_{21}H_{33}N_4O_{13}S_2$ [$M+H^+$] *m/z* 613.1486. Found; 613.1481.

A solution of **13e** (2.95 g, 3.55 mmol) in THF (120 mL) was treated with 1 N HCl (80 mL), and the solution was stirred at 20 °C for 1 h, then diluted with water (100 mL), neutralized with satd. $NaHCO_3$, and extracted with EtOAc (3x80 mL). The combined organic phases were washed with brine and dried, the solvent was evaporated, and the residue was purified by chromatography on silica gel, eluting with EtOAc/MeOH(100:1), to give 2-[{2-[(methylsulfonyl)oxy]ethyl}-4,6-dinitro-6-([{2-hydroxypropyl-amino}carbonyl)anilino]ethyl methanesulfonate (**13d**) (1.4 g, 75%): as a yellow solid: mp (EtOAc/petroleum ether) 130-133 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.74 (d, J = 2.8 Hz, 1H), 8.72 (m, 1H), 8.32 (d, J= 2.8 Hz, 1H), 4.29 (m, 4 H), 3.47 (m, 8 H), 3.14 (s, 6 H), 1.71 (m, 2 H); ^{13}C NMR δ 165.2, 146.3, 145.3, 140.8, 135.9, 127.5, 122.3, 67.3, 58.4, 51.4, 36.8, 36.5, 31.7. Anal. ($C_{16}H_{24}N_4O_{12}S_2$) C, H, N.

Treatment of **13d** (0.25 g, 0.45 mmol) with LiBr (53 mg, 0.7 mmol) in EtOAc (50 mL) at 60 °C for 3 h, and chromatography of the product on silica gel, eluting with

EtOAc/petroleum ether (from 2:1 to 1:0) gave **IIb5** (0.16 g, 66%): as yellow foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.74 (d, J = 2.8 Hz, 1H), 8.73 (m, 1H), 8.31 (d, J= 2.8 Hz, 1H), 4.28 (m, 2 H), 3.65-3.44 (m, 10 H), 3.13 (s, 3 H), 1.70 (m, 2 H); ^{13}C NMR δ 165.1, 145.7, 145.4, 141.0, 136.2, 127.3, 122.1, 67.5, 58.4, 51.1, 36.7, 36.5, 31.7, 29.6. HRMS (FAB) Calcd. For $C_{15}H_{22}{}^{79}BrN_4O_9S$ [M+H$^+$] *m/z* 513.0291. Found; 513.0281.

2-((2-Bromoethyl)-2-{[(2,3-dihydroxypropyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate (IIb6). A solution of 2-(2-({[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]amino}carbonyl){2-[(methylsulfonyl)oxy]ethyl}-4,6-dinitroanilino)ethyl methanesulfonate (**13g**) [Palmer et al., *J. Med. Chem.* 1997, 40, 1272] (5.0 mmol) in MeOH (200 mL) was treated with p-toluenesulfonic acid (0.2 g) at room temperature for 4 h. Most of the MeOH was then evaporated, and the residue was taken up in EtOAc (200 mL), washed with satd. $NaHCO_3$ and brine, dried and concentrated. Chromatography of the product on silica gel, eluting with EtOAc/MeOH (20:1), gave 2-(2-{[(2,3-dihydroxypropyl)amino]carbonyl}{2-[(methylsulfonyl)oxy]ethyl}-4,6-dinitroanilino)ethyl methanesulfonate (**13f**) (2.0 g, 73%): as yellow foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.77 (m, 1 H, CONH), 8.74 (d, J = 3.0 Hz, 1 H, H-5), 8.37 (d, J = 3.0 Hz, 1 H, H-3), 4.30 (m, 4 H, 2xCH_2OMs), 3.66 (m, 1 H), 3.48-3.30 (m, 8 H), 3.14 (s, 6 H, 2xOSO_2CH_3); ^{13}C NMR δ 165.42, 146.24, 145.09, 140.60, 135.77, 127.67, 122.26, 69.77, 67.29, 63.87, 51.29, 42.98, 36.44. HRMS m/z $(M+1)^+$ required for $C_{16}H_{25}N_4O_{13}S_2$ 545.08596; Found 545.08680.
Treatment of **13f** (1.50 g, 2.75 mmol) with LiBr (0.21 g, 2.0 mmol) in EtOAc (60 mL) at 60 °C for 3 h, followed by chromatography on silica gel and elution with EtOAc/MeOH 20:1), gave the dibromide **14f** (0.5 g, 35%) as a yellow foam and then **IIb6** (0.62 g, 34%): yellow solid; ^{1}H NMR [$(CD_3)_2SO$] δ 8.74 (d, *J* = 2.8 Hz, 1 H, H-5), 8.71 (m, 1 H, CONH), 8.36 (d, *J* = 2.8 Hz, 1 H, H-3), 4.28 (m, 2 H, CH_2OMs), 3.69-3.30 (m, 11 H), 3.14 (s, 3 H); ^{13}C NMR δ 165.52, 145.87, 145.30, 140.93, 136.20, 127.64, 122.23, 68.89, 67.62, 63.93, 54.35, 51.08, 43.04, 36.52, 29.80. Anal. Calcd for $C_{15}H_{21}BrN_4O_{10}S$: C, 34.1; H, 4.0; N, 10.6; Br, 15.0. Found: C, 34.0; H, 4.0; N, 10.5; Br, 15.2%. Further elution gave starting material **8e** (0.28, 19%).

2-[(2-Bromoethyl)-2-({[3-(4-morpholinyl)propyl]amino}carbonyl)-4,6-dinitroanilino]ethyl methanesulfonate (IIb7). 2-Chloro-*N*-[3-(4-morpholinyl)propyl]-3,5-dinitrobenzamide (**12h**) (0.5 g, 1.34 mmol) was reacted with diethanolamine (0.5 g) in *p*-dioxane (10 mL) at room temperature for 3 h. The reaction mixture was poured into brine, extracted with EtOAc (3x70 mL), and the combined organic phases were dried and concentrated under reduced pressure to give crude 2-[bis(2-hydroxyethyl)amino]-*N*-[3-(4-morpholinyl)propyl]-3,5-dinitrobenzamide. This was dissolved in CH_2Cl_2 (100 mL), cooled in an ice-bath, and treated with Et_3N (1.5 mL) followed by MsCl (0.7 mL) in one portion. After stirring for 10 min, sat. $NaHCO_3$ (100 mL) was added and the mixture was stirred for a further 30 min, then the aqueous phase was extracted with CH_2Cl_3 (2x70 mL). The combined organic phases were dried and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with EtOAc/MeOH (20:1 to 9:0) to give yield 2-[{2-[(methylsulfonyl)oxy]ethyl}-2-({[3-(4-morpholinyl)propyl]amino}carbonyl)-4,6-dinitroanilino]ethyl methanesulfonate (**13h**) (0.75 g, 93%) as a foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.77 (m, 1H, CONH), 8.74 (d, *J* = 2.7 Hz, 1 H, H-5), 8.20 (d, *J* = 2.7 Hz, 1 H, H-3), 4.28 (m, 4 H, 2x C*H*$_2$OMs), 3.56 (m, 5 H), 3.44 (m, 5 H), 3.15 (s, 6 H), 2.35 (m, 6 H), 1.71 (m, 2 H).

A solution of **13h** (0.70 g, 1.17 mmol) in EtOAc (100 mL) was treated with LiBr (118 mg, 1.36 mmol) at 60 °C for 2 h. Volatiles were removed under reduced pressure, and the residue was adsorbed directly onto silica gel and chromatographed. Elution with EtOAc/MeOH (from 20:1 to 10:1) gave 2-[bis(2-bromoethyl)amino]-N-[3-(4-morpholinyl)propyl]-3,5-dinitrobenzamide (**14h**) 228 mg (34%) as a yellow oil; ^{1}H NMR [$(CD_3)_2SO$] δ 8.77 (m, 1 H, CONH), 8.76 (d, *J* = 2.8 Hz, 1 H, H-5), 8.30 (d, *J* = 2.8 Hz, 1 H, H-3), 3.58-3.42 (m, 14 H), 2.36 (m, 6 H), 1.70 (m, 2 H); ^{13}C NMR δ 165.08, 145.57, 145.27, 141.19, 136.40, 127.27, 122.10, 66.08, 59.66, 55.64, 53.19, 37.61, 25.39, 13.99. HRMS m/z $(M+1)^+$ required for $C_{18}H_{25}{}^{79}Br_2N_5O_6$: 566.0250. Found; 566.0241. Later eluates gave **IIb7** (300 mg, 44%); as yellow foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.77 (m, 1 H, CONH), 8.75 (d, *J* = 2.6 Hz, 1 H, H-4), 8.31 (d, *J* = 2.6 Hz, 1 H, H-6), 4.28 (m, 2 H, CH_2OMs), 3.56 (m, 7 H), 3.44 (m, 5 H), 3.14 (s, 3 H), 2.35(m, 6 H), 1.71 (m, 2 H); ^{13}C NMR δ 165.07, 145.79, 145.31, 140.92, 136.04, 127.36, 122.21, 67.50, 66.09, 59.64, 55.68, 53.21, 51.10, 37.63, 36.45, 25.41, 14.00. HRMS m/z

$(M+1)^+$ required for $C_{19}H_{29}{}^{79}BrN_5O_9S$ 582.08519. Found 582.08694; together with starting material **13h** (117 mg, 18%).

Methyl 3-{[2-((2-chloroethyl){2-[(methylsulfonyl)oxy]ethyl}amino)-3,5-dinitrobenzoyl]amino}propanoate (IIb8). Methyl alanine hydrochloride (2.55 g, 18.3 mmol) was dissolved in water (12 mL), and the solution was diluted with Me_2CO (20 mL) and Et_2O (50 mL). This was then poured into a solution of crude 2-chloro-3,5-dinitrobenzoyl chloride [prepared from 2-chloro-3,5-dinitrobenzoic acid **11** (5.0 g, 18.3 mmol) with $SOCl_2$] in Me_2CO (50 mL) while cooling in an ice-bath. The mixture was stirred for 30 min, then poured into water and extracted with EtOAc. The organic phase was washed with satd. $NaHCO_3$ and brine, dried, and concentrated to give methyl 3-[(2-chloro-3,5-dinitrobenzoyl)amino]propanoate (**12i**) (4.45 g, 73.3%): mp (EtOAc/petroleum ether) 128-130 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.99 (d, J = 2.7 Hz, 1 H, H-4), 8.96 (m, 1 H, CONH), 8.51 (d, J = 2.7 Hz, 1 H, H-6), 3.63 (s, 3 H, CO_2CH_3), 3.50 (m, 2 H, CONHCH_2), 2.64 (m, 2 H, CH_2CO_2). The product was used without further purification.

A mixture of **12i** (2.5 g, 7.6 mmol) and diethanolamine (2.0 g) in *p*-dioxane (30 mL) was kept at room temperature for 3 h, then poured into brine and extracted with EtOAc (3x70 mL). The combined organic phases were dried and evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (15 mL), cooled in an ice-bath, and treated with Et_3N (8 mL) and MsCl (4 mL). After stirring for 10 min, satd. $NaHCO_3$ (100 mL) was added, and following a further 30 min of stirring the aqueous phase was extracted with CH_2Cl_3 (2x70 mL). The combined organic phases were dried and then evaporated under reduced pressure, and the residue was then purified by column chromatography on silica gel. Elution with EtOAc/petroleum ether (1:1 to 1:0) gave **IIb8** (0.2 g, 5%): as yellow oil; ^{1}H NMR [$(CD_3)_2SO$] δ 8.88 (m, 1 H, CONH), 8.74 (d, J = 2.7 Hz, 1 H, H-4), 8.31 (d, J = 2.7 Hz, 1 H, H-6), 4.29 (m, 2 H, CH_2OMs), 3.71 (m, 2 H, CH_2Cl), 3.63 (s, 3 H, CO_2CH_3), 3.54 – 3.36 (m, 6 H), 3.14 (s, 3 H, OSO_2CH_3), 2.65 (m, 2 H, CH_2CO_2); ^{13}C NMR δ 171.68, 165.34, 146.14, 145.17, 140,74, 135.59, 127.58, 122.42, 67.47, 54.22, 51.45, 51.22, 41.37, 36.48, 35.44, 32.95. HRMS m/z $(M+1)^+$ required for $C_{16}H_{22}{}^{35}ClN_4O_{10}S$; 497.0745. Found; 497.0748.

Further elution gave methyl 3-{[2-bis{2-[(methylsulfonyl)oxy]ethyl}amino)-3,5-dinitrobenzoyl]amino}propanoate (**13i**) (2.6 g, 62%): as yellow oil; ^{1}H NMR

$[(CD_3)_2SO]$ δ 8.90 (m, 1 H, CONH), 8.74 (d, $J = 2.7$ Hz, 1 H, H-4), 8.32 (d, $J = 2.7$ Hz, 1 H, H-6), 4.30 (m, 4 H, $2xCH_2OMs$), 3.63 (s, 3 H, CO_2CH_3), 3.52 (m, 2 H, $CONHCH_2$), 3.44 (m, 4 H, 2x CH_2N), 3.14 (s, 6 H, $2xOSO_2CH_3$), 2.65 (m, 2 H, CH_2CO_2); ^{13}C NMR δ 171.66, 165.28, 146.36, 144.98, 140,52, 135.23, 127.64, 122.50, 67.20, 51.40, 51.25, 36.44, 35.45, 32.91. HRMS m/z $(M+1)^+$ required for $C_{17}H_{25}N_4O_{13}S_2$: 557.0860. Found: 557.0853.

In an alternative preparation of **IIb8**, a solution of **13i** (0.417 g, 0.75 mmol) in DMF (10 mL) was treated with LiCl (0.038 g, 1.00 mmol) at 60 °C for 2 h, and then cooled and poured into dilute HCl and extracted with EtOAc (3x80 mL). Workup and chromatography of the product on silica gel, eluting with EtOAc/petroleum ether from 1:1 to 2:1, gave methyl 3-({2-[bis(2-chloroethyl)amino]-3,5-dinitrobenzoyl}amino)propanoate (**15i**) (0.16 g, 51%): as yellow oil; ^{1}H NMR $[(CD_3)_2SO]$ δ 8.85 (m, 1 H, CONH), 8.74 (d, $J = 2.7$ Hz, 1 H, H-4), 8.29 (d, $J = 2.7$ Hz, 1 H, H-6), 3.68 (m, 4 H, 2x CH_2Cl), 3.63 (s, 3 H, CO_2CH_3), 3.50 (m, 2 H, $CONHCH_2$), 3.41 (m, 4 H, $N(CH_2)_2$), 2.64 (m, 2 H, CH_2CO_2); ^{13}C NMR δ 171.59, 165.28, 145.81, 145.31, 140,89, 135.89, 127.45, 122.26, 54.08, 51.40, 41.51, 35.35, 32.92. . Further elution then gave **IIb8** (0.124 g, 33%), identical with the sample prepared above.

Methyl 3-{[2-((2-bromoethyl){2-[(methylsulfonyl)oxy]ethyl}amino)-3,5-dinitrobenzoyl]amino}propanoate (IIb9). Treatment of **13i** (2.04 g, 3.67 mmol) with LiBr (0.318 g, 3.67 mmol) in EtOAc (100 mL) at 60 °C for 3 h, followed by chromatography on silica gel and elution with EtOAc/petroleum ether from 1:1 to 1:0) gave methyl 3-({2-[bis(2-bromoethyl)amino]-3,5-dinitrobenzoyl}amino)propanoate (**14i**) (0.55 g, 29%): as yellow foam; ^{1}H NMR $[(CD_3)_2SO]$ δ 8.86 (m, 1 H, CONH), 8.74 (d, $J = 2.7$ Hz, 1 H, H-4), 8.29 (d, $J = 2.7$ Hz, 1 H, H-6), 3.63 (s, 3 H, CO_2CH_3), 3.60 – 3.43 (m, 10 H), 2.64 (m, 2 H, CH_2CO_2); ^{13}C NMR δ 171.60, 165.28, 145.39, 145.36, 141,07, 136.05, 127.44, 122.25, 53.97, 51.44, 35.35, 32.95, 29.96. HRMS m/z $(M+1)^+$ required for $C_{15}H_{19}{}^{79}Br_2N_4O_7$: 524.9621. Found; 524.9616.

Further elution gave **IIb9** (0.96 g, 48%): as yellow foam; ^{1}H NMR $[(CD_3)_2SO]$ δ 8.89 (m, 1 H, CONH), 8.74 (d, $J = 2.7$ Hz, 1 H, H-4), 8.31 (d, $J = 2.7$ Hz, 1 H, H-6), 4.28 (m, 2 H, CH_2OMs), 3.63 (s, 3 H, CO_2CH_3), 3.60 – 3.43 (m, 8 H), 3.14 (s, 3 H,

OSO_2CH_3), 2.65 (m, 2 H, CH_2CO_2); ^{13}C NMR δ 171.63, 165.28, 145.87, 145.19, 140,81, 135.65, 127.54, 122.37, 67.47, 54.25, 51.42, 51.02, 36.45, 35.40, 32.93, 29.69. HRMS m/z $(M+1)^+$ required for $C_{16}H_{22}{}^{79}BrN_4O_{10}S$: 541.0240. Found; 541.0228, followed by starting material **13g** (0.45 g, 22%).

Example C : Preparation of analogues of class IIc by the method outlined in Scheme 3.

2-[3-(Aminocarbonyl)(2-chloroethyl)-2,4-dinitroanilino]ethyl methanesulfonate (IIc1).

A solution of methyl 3-[bis(2-hydroxyethyl)amino]-2,6-dinitrobenzoate [Palmer et al., *J. Med. Chem.* 1996, 39, 2518] (7.24 g, 22 mmol) in CH_2Cl_2 (120 mL) was cooled in an ice-bath and Et_3N (15 mL) and MsCl (8 mL) were added in one portion. After stirred for 10 min, satd. $NaHCO_3$ (100 mL) was added, and after a further 30 min the aqueous phase was extracted with CH_2Cl_2 (2x70 mL), the combined organic phase were dried, concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel. Elution with EtOAc/petroleum ether (1:1 to 1:0), gave crude methyl 3-(bis{2-[(methylsulfonyl)oxy]ethyl}amino)-2,6-dinitrobenzoate (**16**) (10.67 g, 100%) as a yellow oil; ^1H NMR [$(CD_3)_2SO$] δ 8.32 (d, J = 9.6 Hz, 1 H, H-5), 7.75 (d, J = 9.6 Hz, 1 H, H-4), 4.32 (m, 4 H), 3.88 (s, 3 H), 3.67 (m, 4 H), 3.14 (m, 6 H); ^{13}C NMR δ 163.02, 147.59, 138.40, 136.46, 128.33, 125.83, 123.96, 66.73, 54.00, 50.24, 45.58, 36.58.

Hydrolysis of **16** (10.6 g, 21.9 mmol) with 3 N KOH (40 mL) in dioxane (200 mL) at room temperature for 15 min, followed by acidification with 1 N HCl and extraction with EtOAc, gave a quantitative yield of crude 3-(bis{2-[(methylsulfonyl)oxy]ethyl}amino)-2,6-dinitrobenzoic acid (**17**): mp 200-210 °C, HRMS: $C_{13}H_{18}N_3O_{12}S_2$ requires *m/z* 472.0332. Found: 472.033, that was used without purification. The acid chloride ($SOCl_2$/cat. DMF) from **17** (3.2 g, 6.8 mmol) was dissolved in Me_2CO (30 mL), cooled in an ice-bath and treated with concentrated NH_4OH (10 mL). After stirring for 10 min. the reaction mixture was acidified to pH 2-3 with 1 N HCl, then most of the solvent was evaporated and the residue was partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc

(2x80 mL) and the combined organic extracts were dried and evaporated under reduced pressure. The residue was adsorbed directly onto silica gel and chromatographed. Elution with EtOAc/petroleum ether (from 1:1 to 1:0) gave **IIc1** (0.145 g, 5.2%: mp (EtOAc) 134-136 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.25 (d, J = 9.3 Hz, 1 H, H-5), 8.23 (s, 1 H, NH), 7.89 (s, 1 H, NH), 7.64 (d, J = 9.3 Hz, 1 H, H-6), 4.27 (m, 2 H, CH_2OMs), 3.73 (m, 2 H), 3.66 (m, 2 H), 3.59 (m, 2 H), 3.15 (s, 3 H); ^{13}C NMR δ 163.06, 146.40, 140.52, 137.65, 129.42, 127.51, 122.89, 66.83, 52.93, 50.16, 41.45, 36.57. Anal. Calcd. For $C_{12}H_{15}ClN_4O_8S$: C, 35.1; H, 3.7; N, 13.6; Cl, 8.6. Found: C, 35.5; H, 3.7; N, 13.6; Cl, 8.6%.

Elution of the column with EtOAc/MeOH (50:1) gave 2-(3-(aminocarbonyl){2-[(methylsulfonyl)oxy]ethyl}-2,6-dinitroanilino)ethyl methanesulfonate **(18a)** (1.1 g, 34%): mp (EtOAc/MeOH/petroleum ether) 160-162 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.26 (d, J = 9.3 Hz, 1 H, H-5), 8.23 (s, 1 H, NH), 7.89 (s, 1 H, NH), 7.66 (d, J = 9.3 Hz, 1 H, H-6), 4.27 (m, 4 H, 2x-CH_2OMs), 3.63 (m, 4 H, 2x-CH_2N), 3.15 (s, 6 H, 2x CH_3SO_3-); ^{13}C NMR δ 163.00, 146.51, 140.98, 137.99, 129.30, 127.47, 123.40, 66.74, 50.44, 36.56. Anal. Calcd. For $C_{13}H_{18}N_4O_{11}S_2$: C, 33.2; H, 3.9; N, 11.9. Found: C, 33.5; H, 3.8; N, 11.9%.

2-[3-(Aminocarbonyl)(2-bromoethyl)-2,6-dinitroanilino]ethyl methanesulfonate (IIc2). LiBr (117 mg, 1.34 mmol) was added in one portion to a solution of **18a** (0.474 g, 1.0 mmol) in Me_2CO/EtOAc (1:1, 100 mL), and the reaction mixture was heated to 60 °C for 2 h. Volatiles were removed under reduced pressure, and the residue was adsorbed directly onto silica gel and chromatographed. Elution with EtOAc/petroleum ether (1:1) gave 3-[bis(2-bromoethyl)amino]-2,6-dinitrobenzamide **(19a)** (95 mg, 21%): as a yellow oil; ^{1}H NMR [$(CD_3)_2SO$] δ 8.25 (d, J = 9.5 Hz, 1 H, H-5), 8.22 (s, 1 H, NH), 7.88 (s, 1 H, NH), 7.63 (d, J = 9.5 Hz, 1 H, H-4), 3.68 (m, 4 H), 3.58 (m, 4 H(Lit. [Palmer et al., J. Med. Chem., 1996, 39, 2518-2528].

Further elution with EtOAc/petroleum ether (3:1) gave **IIc2** (208 mg, 46%): mp (EtOAc/petroleum ether) 143-145 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.25 (d, J = 9.3 Hz, 1 H, H-5), 8.23 (s, 1 H, NH), 7.89 (s, 1 H, NH), 7.64 (d, J = 9.3 Hz, 1 H, H-6), 4.28 (m, 2 H, CH_2OMs), 3.67 (m, 4 H), 3.57 (m, 2 H), 3.16 (s, 3 H); ^{13}C NMR δ 163.05, 146.17, 140.49, 137.68, 129.42, 127.53, 122.89, 66.85, 52.92, 50.04, 36.57, 29.95. Anal. Calcd.

For $C_{12}H_{15}BrN_4O_8S$: C, 31.7; H, 3.3; N, 12.3; Br, 17.4. Found: C, 31.9; H, 3.3; N, 12.2; Br, 17.5%.

Later eluates gave starting material **18a** (150 mg).

2-((2-Bromoethyl)-3-{[(2-hydroxyethyl)amino]carbonyl}-2,6-dinitroanilino)ethyl methanesulfonate (IIc3). Treatment of 3-(3-{[(2-hydroxyethyl)amino]carbonyl}{3-[(methylsulfonyl)oxy]butyl}-2,4-dinitroanilino)-1-methylpropyl methanesulfonate (**18b**)] (310 mg, 0.6 mmol) in EtOAc (50 mL) with LiBr (78 mg, 0.9 mmol), followed by chromatography on silica gel and elution with EtOAc/petroleum ether (from 1:1 to 1:0) gave 3-[bis(2-bromoethyl)amino]-*N*-(2-hydroxyethyl)-2,6-dinitrobenzamide (**19b**) (70 mg, 25%) as a foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.80 (m, 1H, CONH), 8.24 (d, J= 9.4 Hz, 1H), 7.63 (d, J=9.4 Hz, 1H), 4.66 (m, 1 H), 3.70 (m, 4 H), 3.60 (m, 4 H), 3.45 (m, 2 H), 3.22 (m, 2 H); ^{13}C NMR δ 161.4, 145.8, 140.2, 137.5, 129.2, 127.6, 122.6, 59.0, 52.6, 41.7, 30.0.. HRMS (FAB) Calcd. For $C_{13}H_{17}{}^{79}Br_2N_4O_6$ [M+H$^+$] *m/z* 482.9515. Found; 482.9508.

Further elution with EtOAc/MeOH (50:2) gave **IIc3** (118 mg, 39%): mp. 94-97 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.80 (m, 1H, CONH), 8.25 (d, J= 9.4 Hz, 1H), 7.64 (d, J=9.4 Hz, 1H), 4.67 (m, 1 H), 4.27 (m, 2 H), 3.63 (m, 4 H), 3.57 (m, 2 H), 3.45 (m, 2 H), 3.26 (m, 2 H), 3.15 (s, 3 H); ^{13}C NMR δ 161.4, 146.2, 140.5, 137.7, 129.2, 127.5, 122.9, 66.8, 59.0, 50.0, 41.7, 36.6, 29.9. Anal. ($C_{14}H_{19}BrN_4O_9S$) C, H, N.

2-((2-Chloroethyl)-3-{[(3-hydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate (IIc4). Reaction of the acid chloride of **17** with 3-aminopropanol in Me_2CO at 0 °C as described above, followed by chromatography of the product on silica gel and elution with EtOAc/petroleum ether (1:1), gave **IIc4** (292 mg, 12%): mp (EtOAc/petroleum ether) 104-109 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.75 (t, J = 5.8 Hz, 1 H, CONH), 8.24 (d, J = 9.4 Hz, 1 H, H-5), 7.64 (d, J = 9.4 Hz, 1 H, H-6), 4.44 (m, 1 H, CHO*H*), 4.26 (m, 2 H), 3.72 (m, 2 H), 3.65 (m, 2 H), 3.59 (m, 2 H), 3.43 (m, 2H), 3.20 (m, 2 H), 3.15 (s, 3 H), 1.60 (m, 2 H); ^{13}C NMR δ 161.09, 146.42, 140.49, 137.65, 129.23, 127.58, 122.91, 66.82, 58.22, 52.88, 50.11, 41.44, 36.57, 36.37, 31.57. Anal. Calcd. For $C_{15}H_{21}ClN_4O_9S$: C, 38.5; H, 4.5; N, 12.0; Cl, 7.5. Found: C, 38.8; H, 4.8; N, 11.5; Cl, 7.0%.

Further elution with EtOAc gave 2-(3-{[(3-hydroxypropyl)amino]carbonyl}{2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilino)ethyl methanesulfonate (**18e**) (1.1g, 41%): mp (EtOAc/MeOH/petroleum ether) 160-162 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.77 (t, J = 5.8 Hz, 1 H, CONH), 8.26 (d, J = 9.4 Hz, 1 H, H-5), 7.66 (d, J = 9.4 Hz, 1 H, H-6), 4.43 (m, 1 H, CHO*H*), 4.27 (m, 4 H, 2xC*H*$_2$OMs), 3.63 (m, 4 H, 2xCH_2N), 3.43 (m, 2 H), 3.20 (m, 2 H), 3.15 (s, 6 H, 2xCH_3SO_3), 1.60 (m, 2 H); ^{13}C NMR δ 161.03, 146.52, 140.95, 138.00, 129.12, 127.54, 123.42, 66.72, 58.22, 50.39, 36.55, 36.37, 31.57. Anal. Calcd. For $C_{16}H_{24}N_4O_{12}S_2$: C, 36.4; H, 4.6; N, 10.6. Found: C, 36.6; H, 4.5; N, 10.6%.

2-((2-Bromoethyl)-3-{[(3-hydroxypropyl)amino]carbonyl}-2,6-dinitroanilino)ethyl methanesulfonate (IIc5). Treatment of **18c** (716 mg, 1.36 mmol) in EtOAc (200 mL) with LiBr ((175 mg, 2.0 mmol) as above, followed by chromatography on silica gel and elution with EtOAc/ etroleum ether (from 1:1 to 1:0) gave 3-[bis(2-bromoethyl)amino]-*N*-(3-hydroxypropyl)-2,6-dinitrobenzamide (**19c**) (289 mg, 42%) as a foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.75 (t, J = 5.8 Hz, 1 H, CONH), 8.23 (d, J = 9.4 Hz, 1 H, H-5), 7.62 (d, J = 9.4 Hz, 1 H, H-4), 4.47 (m, 1 H, CHO*H*), 3.68 (m, 4 H), 3.57 (m, 4 H), 3.43 (m, 2 H), 3.20 (m, 2 H), 1.60 (m, 2 H); ^{13}C NMR δ 161.20, 146.90, 140.20, 137.53, 129.36, 127.69, 122.56, 58.29, 52.64, 36.42, 31.61, 30.13. HRMS m/z $(M+1)^+$ required for $C_{14}H_{19}{}^{79}Br_2N_4O_6$: 496.9671. Found: 496.9667.

Further elution with EtOAc/MeOH (50:2) gave **IIc5** (270 mg, 39%): mp. 115-117 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.75 (t, J = 5.8 Hz, 1 H, CONH), 8.24 (d, J = 9.4 Hz, 1 H, H-5), 7.64 (d, J = 9.4 Hz, 1 H, H-6), 4.43 (m, 1 H, CHO*H*), 4.27 (m, 2 H, C*H*$_2$OMs), 3.66 (m, 4 H, 2xCH_2N), 3.59 (m, 2 H), 3.44 (m, 2 H), 3.22 (m, 2 H), 3.15 (s, 3 H, CH_3SO_3), 1.60 (m, 2 H); ^{13}C NMR δ 161.08, 146.19, 140.47, 137.69, 129.24, 127.59, 122.91, 66.83, 58.22, 52.87, 50.00, 36.57, 36.37, 31.58, 29.95. Anal. Calcd. For $C_{15}H_{21}BrN_4O_9S$: C, 35.2; H, 4.1; N, 10.9; Br, 15.4. Found: C, 35.4; H, 3.9; N, 11.0; Br, 16.3%.

2-((2-Bromoethyl)-3-{[(4-hydroxybutyl)amino]carbonyl}-2,6-dinitroanilino)ethyl methanesulfonate (IIc6). Treatment of 3-(3-{[(4-hydroxybutyl)amino]carbonyl}{3-[(methylsulfonyl)oxy]butyl}-2,4-dinitroanilino)-1-methylpropyl methanesulfonate **(18d)** (500 mg, 0.92 mmol) in EtOAc (100 mL) with LiBr (110 mg, 1.4 mmol), followed by chromatography on silica gel and elution with EtOAc/petroleum ether (from 1:1 to 1:0) gave 3-[bis(2-bromoethyl)amino]-*N*-(4-hydroxybutyl)-2,6-dinitrobenzamide (**19d**) (100

mg, 21%) as a foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.73 (m, 1H, CONH), 8.25 (d, J= 9.4 Hz, 1H), 7.63 (d, J=9.4 Hz, 1H), 4.38 (m, 1 H), 3.69 (m, 4 H), 3.57 (m, 4 H), 3.40 (m, 2 H), 3.14 (m, 2 H), 1.47 (m, 4 H); ^{13}C NMR δ 161.0, 145.8, 140.2, 137.6, 129.3, 127.6, 122.6, 60.2, 52.6, 30.0, 29.6, 24.8. HRMS (FAB) Calcd. For $C_{15}H_{20}{}^{79}Br_2N_4O_6$ [$M+H^+$] *m/z* 510.9828. Found; 510.9819.

Further elution with EtOAc/MeOH (50:2) gave **IIc6** (117 mg, 30%): mp. 114-117 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.74 (m, 1 H, CONH), 8.25 (d, J = 9.4 Hz, 1 H), 7.65 (d, J =9.4 Hz, 1 H), 4.37 (m, 1 H), 4.27 (m, 2 H), 3.65 (m, 4 H), 3.57 (m, 2 H), 3.35 (m, 2 H), 3.16 (m, 2 H), 3.15 (s, 3 H), 1.47 (m, 4 H); ^{13}C NMR δ 160.0, 146.1, 140.6, 137.8, 129.2, 127.5, 122.9, 66.8, 60.2, 52.9, 50.0, 36.6, 29.9, 29.6, 24.9. Anal. ($C_{16}H_{23}BrN_4O_9S$) C, H, N.

2-((2-Chloroethyl)-3-{[(2,3-dihydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate (IIc7). Reaction of the acid chloride of **17** (2.4 g, 5.1 mmol) with 3-amino-1,2-propanediol Me_2CO at 0 °C as described above, followed by chromatography of the product on silica gel and elution with EtOAc, gave **IIc7** (240 mg, 10%): mp (EtOAc) 100-105 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.77 (t, J = 5.8 Hz, 1 H, CONH), 8.24 (d, J = 9.4 Hz, 1 H, H-5), 7.64 (d, J = 9.4 Hz, 1 H, H-6), 4.72 (d, J = 4.9, 1 H, CHO*H*), 4.52 (t, J = 5.7, 1 H, CH_2OH), 4.27 (m, 2 H, CH_2OMs), 3.74 – 3.50 (m, 10 H), 3.15 (s, 3 H, CH_3SO_3), 3.04 (m, 1 H); ^{13}C NMR δ 161.48, 146.38, 140.55, 137.73, 129.28, 127.51, 122.88, 69.89, 66.83, 63.57, 52.95, 50.17, 42.55, 41.43, 36.58. Anal. Calcd. For $C_{15}H_{21}ClN_4O_{10}S$: C, 37.2; H, 4.4; N, 11.6; Cl, 7.2. Found: C, 38.0; H, 4.5; N, 11.1; Cl, 7.2%.

Further elution with EtOAc/MeOH (50 : 1) gave 2-(3-{[(2,3-dihydroxypropyl)amino]carbonyl}{2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilino)ethyl methanesulfonate **(18e)** (480 mg, 51%): mp (MeOH/EtOAc) 60-63 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.78 (t, J = 5.8 Hz, 1 H, CONH), 8.24 (d, J = 9.4 Hz, 1 H, H-5), 7.66 (d, J = 9.4 Hz, 1 H, H-6), 4.72 (d, J = 4.9, 1 H, CHO*H*), 4.52 (t, J = 5.7, 1 H, CH_2OH), 4.27 (m, 4H, 2xCH_2OMs), 3.63 (m, 4 H), 3.52 – 3.30 (m, 5 H), 3.15 (s, 3 H, 2xCH_3SO_3), 3.06 (m, 1 H); ^{13}C NMR δ 161.43, 146.49, 141.01, 138.07, 129.15, 127.47,

123.36, 69.89, 66.73, 63.67, 50.44, 42.55, 36.56. Anal. Calcd. For $C_{16}H_{24}N_4O_{13}S_2$: C, 35.3; H, 4.5; N, 10.3. Found: C, 35.8; H, 4.5; N, 10.5%..

2-((2-Bromoethyl)-3-{[(2,3-dihydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate (IIc8). Treatment of **18e** (0.92 g, 1.7 mmol) in EtOAc (200 mL) with LiBr (170 mg, 1.95 mmol) as above, followed by chromatography on silica gel and elution with EtOAc/MeOH (50:1), gave 3-[bis(2-bromoethyl)amino]-*N*-(2,3-dihydroxypropyl)-2,4-dinitrobenzamide **(19e)** (155 mg, 18%) as yellow oil; ^{1}H NMR [$(CD_3)_2SO$] δ 8.76 (t, J = 5.8 Hz, 1 H, CONH), 8.23 (d, J = 9.5 Hz, 1 H, H-5), 7.63 (d, J = 9.5 Hz, 1 H, H-6), 4.72 (d, J = 5.1 Hz, 1 H, CHO*H*), 4.52 (t, J = 5.7 Hz, 1 H, CH_2OH), 3.70 – 3.50 (m, 11 H), 3.04 (m, 1 H). HRMS m/z $(M+1)^+$ required for $C_{14}H_{19}{}^{79}Br_2N_4O_7$: 512.9621. Found; 512.9603.
Further elution gave **IIc8** (278 mg, 31%): mp (EtOAc) 108-110 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.77 (t, J = 5.8 Hz, 1 H, CONH), 8.24 (d, J = 9.4 Hz, 1 H, H-5), 7.64 (d, J = 9.4 Hz, 1H, H-6), 4.72 (d, J = 4.9, 1 H, CHO*H*), 4.52 (t, J = 5.7, 1 H, CH_2OH), 4.27 (m, 2 H, CH_2OMs), 3.70 – 3.50 (m, 10 H), 3.15 (s, 3 H, CH_3SO_3), 3.06 (m, 1 H); ^{13}C NMR δ 161.47, 146.16, 140.52, 137.77, 129.28, 127.53, 122.88, 69.89, 66.84, 63.57, 52.94, 50.05, 42.55, 36.58, 29.94. Anal. Calcd. For $C_{15}H_{21}BrN_4O_{10}S$: C, 34.1; H, 4.0; N, 10.6; Br, 15.0. Found: C, 34.3; H, 4.1; N, 10.4; Br, 15.4%.
And starting material (200 mg, 22%)

2-[(2-Chloroethyl)-3-({[3-(4-morpholinyl)propyl]amino}carbonyl)-2,4-dinitroanilino]ethyl methanesulfonate (IIc9). Reaction of the acid chloride from **17** (1.3 g) in Me_2CO with 3-(4-morpholinyl)propylamine (1.0 mL) at 0°C as described above, followed by chromatography of the product on silica gel and elution with EtOAc/MeOH (9:1 to 4:1), gave **IIc9** (0.37 g, 25%): mp (EtOAc/petroleum ether) 113-116 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.79 (t, J = 5.6 Hz, 1 H, CONH), 8.25 (d, J = 9.4 Hz, 1 H, H-5), 7.65 (d, J = 9.4 Hz, 1 H, H-6), 4.28 (t, J = 5.3, 2 H), 3.73 (t, J = 6.3, 2 H), 3.66 (t, J = 5.2, 2 H), 3.60 (t, J = 5.9, 2 H), 3.56 (m, 4H), 3.17 (m, 5 H), 2.34 (m, 6H), 1.61 (m, 2H); ^{13}C NMR δ 161.07, 146.44, 140.44, 137.62, 129.23, 127.60, 122.92, 66.81, 66.12, 55.40, 53.19, 52.85, 50.10, 41.45, 37.30, 36.56, 25.12. HRMS m/z $(M+1)^+$ requires $C_{19}H_{29}{}^{35}ClN_5O_9S$: 538.13745. Found: 538.13869.

Later eluates gave 2-[{2-[(methylsulfonyl)oxy]ethyl}-3-({[3-(4-morpholinyl)propyl]amino}carbonyl)-2,4-dinitroanilino]ethyl methanesulfonate **(18f)** (0.93 g, 56%) as a yellow solid, mp (EtOAc/petroleum ether) 90-95 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.79 (t, J = 5.7 Hz, 1 H, CONH), 8.25 (d, J = 9.4 Hz, 1 H, H-5), 7.65 (d, J = 9.4 Hz, 1 H, H-6), 4.28 (t, J = 5.3, 4 H), 3.64 (t, J = 5.2, 4 H), 3.55 (t, J = 4.6, 4 H), 3.15 (m, 8 H), 2.34 (m, 6 H), 1.61 (m, 2 H); ^{13}C NMR δ 161.03, 146.55, 140.90, 137.97, 129.10, 127.56, 123.43, 66.72, 66.12, 55.39, 53.19, 50.37, 37.29, 36.55, 25.13. HRMS m/z $(M+1)^+$ requires $C_{20}H_{32}N_5O_{12}S_2$: 598.14889. Found: 598.14894.

2-[(2-Bromoethyl)-3-({[3-(4-morpholinyl)propyl]amino}carbonyl)-2,4-dinitroanilino]ethyl methanesulfonate (IIc10). LiBr (107 mg, 1.3 mmol) was added in one portion to a warm solution of **18f** (0.53 g, 0.89 mmol) in EtOAc (50 mL). The reaction mixture was heated to 60 °C for 2 h, then volatiles were removed under reduced pressure, and the residue was adsorbed directly onto silica gel and chromatographed. Elution with EtOAc/MeOH (10:1 to 5:1) gave 3-[bis(2-bromoethyl)amino]-*N*-[3-(4-morpholinyl)propyl]-2,6-dinitrobenzamide **(19f)** (109 mg, 22%) as a foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.77 (t, J = 5.6 Hz, 1 H, CONH), 8.23 (d, J = 9.4 Hz, 1 H, H-5), 7.63 (d, J = 9.4 Hz, 1 H, H-6), 3.68 (m, 4H), 3.57 (m, 8 H), 3.17 (m, 2 H), 2.34 (m, 6 H), 1.61 (m, 2 H). HRMS: $C_{15}H_{11}{}^{79}Br_2N_4O_5$ requires *m/z* 438.9253. Found: 438.9228.

Later eluates gave **IIc10** (293 mg, 57%): mp (EtOAc/petroleum ether) 114-117 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.79 (t, J = 5.6 Hz, 1 H, CONH), 8.25 (d, J = 9.4 Hz, 1 H, H-5), 7.65 (d, J = 9.4 Hz, 1 H, H-6), 4.28 (t, J = 5.2, 2 H), 3.66 (m, J = 5.2, 4H), 3.56 (m, J = 4.6, 6 H), 3.17 (m, 5 H), 2.34 (m, 6 H), 1.61 (m, 2 H); ^{13}C NMR δ 161.07, 146.22, 140.39, 137.65, 129.21, 127.62, 122.92, 66.83, 66.07, 55.37, 53.15, 52.83, 49.99, 37.28, 36.57, 29.97, 25.07. HRMS m/z $(M+1)^+$ requires $C_{19}H_{29}{}^{79}BrN_5O_9S$: 582.08694. Found: 582.08639.

Later eluates gave starting material **18f** (124 mg, 23%).

The following Tables 2 and 3 give biological data for the compounds listed in Table 1.

Table 2. Relative cytotoxicities of selected examples of the compounds of Table 1 in NTR-transfected cell lines (18 h exposure).

No	Human ovarian[a]			Human colon[b]		
	IC_{50}[d]		Ratio[e]	IC_{50}[d]		Ratio[e]
	NR-	NR+		NR-	NR+	
Examples of formula IIa						
IIa2	226	0.84	280	150	0.69	235
IIa3	135	0.19	715	80	0.22	387
IIa4	97	0.33	311	70	0.41	172
IIa5	286	0.61	470	192	0.77	250
IIa6				453	5.0	91
IIa7	1110	1.74		804	3.0	268
Examples of formula Iib						
IIb1	80	0.04	1890	20	0.07	303
IIb2	6.0	0.007	762	4.3	0.02	227
IIb3	5.2	0.04	142	3.7	0.06	66
IIb4				9[f]	0.29[f]	31[f]
IIb5				2.9[f]	0.25[f]	12[f]
IIb6	26	0.19	140	11	0.21	52
IIb7	3.1	0.03	102	0.89	0.05	22
IIb8	9.7	0.19	51	4.3	0.36	13
IIb9	3.7	0.15	25	1.44	0.24	6.3
Examples of formula IIc						
IIc1	196	0.55	390	121	1.0	120
IIc2	150	0.21	724	85	0.31	271
IIc3				425[f]	5.1[f]	83[f]
IIc4	800	1.6	549	392	2.6	215
IIc5	280	0.57	497	209	0.85	301
IIc6				314[f]	20[f]	16[f]
IIc7	1680	6.6	267	856	4.3	262
IIc8	890	1.8	509	214	1.5	141
IIc9	433	32	14	262	31	8.3
IIc10	156	11	14	94	11	8.2

Footnotes for Table 2

[a]Human ovarian: wild-type (NR-) is SKOV3, transfected (NR+) is SC3.2.

[b]Human colon: wild-type (NR-) is WIDR, transfected (NR+) is WC14.10.

[c]Chinese hamster fibroblast: wild-type (NR-) is T-78-1, transfected (NR+) is T79-A3.

[d]IC_{50}: the concentration of drug (in micromolar) required to reduce cell numbers to 50% of controls at the end of the evaluation period.

[e]Ratio = IC_{50}(NR-)/IC_{50}(NR+).

[f]4 h exposure.

Table 3. Relative cytotoxicities of selected examples of the compounds of Table 1 in oxic and anoxic tumour cells.

No	IC_{50}s in A549 human lung carcinoma cells (4h exposure) in μM[a]			
	WT[b] anoxic	WT Ratio[c]	P450R[d] anoxic	P450R ratio[c]
Examples of formula IIa				
IIa5	139	5.2	73	9.9
IIa6	348	2.4	31	18
Examples of formula IIb				
IIb3	2.3	26	0.28	133
IIb4	4.5	7	0.45	95
IIb5	4	19	0.34	150
Examples of formula IIc				
IIc3	28	20	2.9	146
IIc4	82	26	7.3	108
IIc5	52	30	4.5	134

Footnotes for Table 3. [a]Human lung carcinoma line.

[b]Wild-type.

[c]Ratio = IC_{50}(aerobic)/IC_{50}(anoxic).

[d]A549 transfected with human cytochrome P450 reductase (P450R).

It is clear from the data of Tables 2 and 3 that the examples of the nitroaniline derivatives of the invention include compounds which are active as cytotoxic agents, and which have the additional capability of being reductively activated by the *E. coli* NTR and/or by endogenous reductase enzymes under hypoxia.

Wherein the foregoing description reference has been made to reagents, or integers having known equivalents thereof, then those equivalents are herein incorporated as if individually set forth.

While this invention has been described with reference to certain embodiments and examples, it is to be appreciated that further modifications and variations may be made to embodiments and examples provided without departing from the scope of the invention.